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A PLANNED CURRICULUM FOR TEACHING HISTOLOGIC TECHNIC*

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The lack of planned curriculum in the training of medical technologists in the field of histologic technic has always presented great difficulties in obtaining adequately trained laboratory personnel. A curriculum in which we are constantly aware of the need of preparation for an exacting profession will create in a student the fine perceptions that produce original work and make him a valuable assistant to the pathologist. Since the end product of a given procedure is indispensable to the pathologist in making the diagnosis, all steps in the technique are essential teaching.

Several factors must be considered in the planning of a curriculum for instruction in histologic technic for students of medical technology. Perhaps the most important of these factors is wise use of the available time in the laboratory. This can be accomplished by several means; weekly lecture periods for the full year during which instruction is given on the theory of histologic technic; the proper choice of various specialized procedures, and, by practical application in a supervised laboratory in which the didactic material is correlated with application.

The arrangement of such a curriculum is the choice of the individual teaching supervisor, but should cover the minimum requirements as outlined in "A Curriculum for Schools of Medical Technology" by Dr. Israel Davidsohn and Dr. Kurt Stern. Although the outline is comprehensive, the means of presenting this training to the student varies greatly from one laboratory to another. The student should have opportunity to learn by instruction, demonstration, systematic practice and study.

The first consideration in the preparation of a good histologic preparation is the fixation of the tissue. This is necessary in order that the structure of the cell shall remain unaltered or shall, at least, preserve certain microscopic features of the original cell. The precipitation or coagulating powers of the reagents employed, as well as pene-

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tration and pH are critical characteristics of a fixing fluid. This information must be a part of the student training program, as well as the compatibility of fixatives with staining methods in which some fixing agents act as mordants for a particular staining process, others inhibit certain stains. The student must know the function of fixing agents, the chemical balance of the fixative ingredients and the advantages, disadvantages and indications for the fixatives used in histopathologic laboratories. If he places a liver biopsy on which a Best carmine stain is requested, in an aqueous fixative, the technique may be precisely accurate, but the glycogen will not be demonstrated. This subject is covered in three lecture periods.

Decalcification of tissue to produce a good section demonstrating nuclear and cytoplasmic detail, either by simple solution of the calcium in a dilute mineral acid, simple solution aided by ion exchange resins, or electrophoretic removal of the calcium ions by the use of an electric current should be covered in the curriculum. The student is given the criteria through which she judges good decaldification. These are, in brief, speed and completeness of decalcification, preservation of cellular detail and nonimpairment of subsequent staining techniques. The complete process, fixation, decalcification, determination of end-point, neutralization of acid, washing and staining technic should be a part of the practice material given to the student. He should see that the determination of the end point by the insertion of a needle damages the tissue, testing by flexibility is unreliable and should learn to use a chemical test for calcium in the following manner. Five ml. of decalcifying fluid are nearly neutralized with $\frac{N}{2}$ sodium hydroxide, then 1 ml. of a five percent solution of sodium or ammonium oxalate is added. Turbidity of the fluid indicates that the decalcifying fluid is free of calcium. While calcium is still present in the tissue, some calcium ions will be dissolved in the decalcifying fluid; therefore, when it is free of calcium, the decalcification is complete. The student learns that the fluid must be changed each time it is tested and several hours must elapse between tests to allow for solution of the calcium.

The next three classroom periods cover instruction in the processing of tissues. The function, advantages, disadvantages and indications for the use of various dehydrants, clearing agents and embedding media must be subject matter on which the student is well informed. For instance, the finished slide for the demonstration of fat cells may present a false negative reaction if the tissue has been priorly treated with any of the numerous chemical agents used in the dehydration and clearing of tissue which dissolve the lipid content and leave behind only the delicate protoplasmic envelopes.

The care and use of a microtome, a microscope, a microtome knife and the technic and difficulties of tissue sectioning are included in the next four lectures, as well as the indications, use, advantages and disadvantages of various types of mounting media. In the last lecture of this series, on mounting media, the student is acquainted with the aqueous media for material which is unstained, that which is stained for fat or metachromatically stained, and the resinous media used for most staining techniques. The aqueous media are of three types, syrups, gelatin and gum arabic media. With the various types of aqueous

media, the student learns how permanent mounts are made by the application of a ringing medium at the edge of the cover slip. This may be done with paraffin wax, colophonium resin with paraffin wax, household cement, asphaltum varnish or clear finger nail polish.

The lectures on the staining of tissue should cover both the physical and chemical theories. The physical theory of simple solubility is well demonstrated by the methods for staining fat, where the stain is more soluble in the fat than in the solvent in which it is dissolved. Surface adsorption, a physical theory, depends on knowing the isoelectric point of the protein involved as well as the pH of the dye solution, since it is affected by the pH of both substances. The actual chemical combination of dye and tissue component comes to us from the early workers in the field of staining. While it is generally true that acid dyes stain the basophilic elements in the cytoplasm and basic dyes stain the acidophilic material in the nucleus, the student must know that hematoxylin, an acid dye, does not stain the cytoplasm, but, in the presence of a mordant is a widely used nuclear stain. Furthermore, no matter how dilute a stain used, the staining solution is never completely decolorized by the tissue, and, if the staining were purely a chemical reaction, it would continue until one of the reagents were exhausted. It is generally accepted, therefore, that the true theory of staining of tissue probably lies in a combination of physical theory of adsorption and the chemical theories.

The lectures on staining should include the principles of dye chemistry, biological stains and dyes, and the methods for using them. Before discussing the chemistry of various stains and reagents it is important to define the various methods by which they are employed. Impregnation, the deposition of the salts of heavy metals over certain cell and tissue structures cannot be classified as staining because the structures demonstrated are rendered opaque and black, the coloring matter is particulate and the deposit is on or around, but not in the element so demonstrated. An element may be broadly defined as stained when, following treatment with a reagent or series of reagents, it acquires color. Staining techniques are divided into histochemical reactions, fat stains and non vital staining, with the majority of staining techniques falling into the non vital staining group.

The classification of dyes, the mordants more commonly used in histological technique, aluminum and ferric salts and alums, and why they are used, is necessary theory for the accomplishment of good histologic technique. The combination of the dye and the mordant forms a compound called a lake, which is then capable of attaching itself firmly to the tissue, as in hematoxylin stains. The student should also know that some lakes are unstable and require that the tissue be mordanted immediately before staining, as in Heidenhain's iron hematoxylin, or even afterwards, as in the Gram technic. The teaching should include the difference between mordants and accentuators, which do not form a lake with the dye, do not have an obvious chemical union but increase the selectivity of the staining power of the dye which would be capable of staining without the accentuator. The phenol in carbon thionin and carbol fuchsin and the potassium hydroxide in Loeffler's methylene blue are accentuators.

The staining reaction known as metachromasia, in which certain tissue components in the presence of certain dyes of the coal-tar group, will stain a color other than the basic color of the dye, should be part of this teaching since many of the techniques for the demonstration of amyloid and mucin use metachromatic dyes. Amyloid will stain red with methyl violet, while the rest of the tissue will stain violet and mucin will stain red with toluidine blue with the rest of the tissue staining shades of blue. In addition to mucin and amyloid, cartilage and mast cell granules exhibit metachromasia. The most important metachromatic dyes are thionin, methylene blue, toluidine blue, safranin and methyl violet.

The theory and methods of special staining are covered over a period of eleven lectures. This series of lectures begins with the four basic processes by which connective tissue fibers are demonstrated, silver impregnation from alkaline solution, staining with acid anilin dyes from strongly acid solution, phosphotungestic and phosphomolybdic acid hematoxlyin methods and the periodic acid leucofuchsin method. Many of the techniques are demonstrated during lecture period with photomicrographs to give the student a comparative picture of what he should accomplish in staining slides.

The histochemistry of polysaccharides and the periodic acid Schiff technics comprise the material for the next lecture period. The chemical basis of the reaction, the essential principles of other oxidizing agents, lead tetraacetate, chromic acid and potassium permanganate as well as the technique of oxidation methods, identification of aldehyde and the use of the sulfite rinse are included in this period.

The theory, methods and demonstration with photomicrographs in the next seven lectures cover bacteria, fungi and inclusion bodies; cytoplasmic granules; hematologic elements; fats and lipids; carbohydrates and mucoproteins; pigments and minerals and nerve cells and fibers.

Although an infant in the field of medical technology, exfoliative cytology should be an integral portion of the curriculum. Our 1958-59 planned curriculum includes this instruction in three lectures, an introduction to exfoliative cytology; normal and atypical cytology and the criteria of malignancy. The planning of laboratory time should include a period in which the student is given instruction in the reading of smears.

A session on the practical application of special stains to diagnosis, although not essential, is of interest to the student and gives him an insight into his professional importance to the pathologist in the complexity of diagnostic achievement.

Discussion

The following outline of a planned curriculum has been in use for one year. It has achieved, at least in part, the intended goal. It is given to you as a guide in your planning and not as a product of perfection.

Lecture Series

1. Fixation; primary fixing agents, modifiers and acids
2. General observations on fixation
3. The use of fixatives in relation to their effect on staining
4. Decalcification

Examination

5. Processing of tissues—the use, advantages and disadvantages of dehydrants
6. Processing of tissues—the use, advantages and disadvantages of clearing agents
7. Processing of tissues—embedding media; frozen sections

Examination

8. The care and use of a microtome and a microscope
9. The care and use of a microtome knife; sharpening of microtome knives
10. The technic and difficulties of tissue sectioning
11. The indication, use, advantages and disadvantages of various types of mounting media

Examination

12. The cell, its component parts, constituents and products
13. The theory of staining
14. The theory of staining (continued)
15. Routine hematoxylin and eosin stains

Examination

16. Connective tissue fiber stains—diammine silver hydroxide reticulum methods; diammine silver carbonate reticulum methods
17. Connective tissue fiber stains—the hematoxylin methods; the periodic acid Schiff methods; the acid anilin dye methods
18. Connective tissue fiber stains—the phosphomolybdic and phosphotungstic acid methods; the single solution methods; the hydrochloric acid methods; the elastic fiber methods

Examination

19. The theory and methods of the periodic acid leucofuchsin technics
20. The theory and methods for staining bacteria, fungi and inclusion bodies
21. Staining of cytoplasmic granules
22. Staining of hematologic elements

Examination

23. Staining of fats and lipids
24. Stains for carbohydrates and mucoproteins
25. Stains for pigments and minerals
26. Stains for nerve cells and fibers—special neurohistological techniques

Examination

27. Introduction to exfoliative cytology
28. Normal and atypical cytology
29. Criteria of malignancy in reference to exfoliative cytology

Examination

30. The application of special stains to diagnosis
31. Review of fixation, dehydration, clearing, embedding agents, mounting media and cutting of sections
32. Review of the theory of staining and routine staining methods

33. Review of the forty-one special staining techniques the student performs in the laboratory

Final Comprehensive Examination

Techniques Done By the Student in the Laboratory

Stains	Number of Different Methods
Connective tissue fiber stains	
Diammine silver hydroxide reticulum methods	3
Diammine silver carbonate reticulum methods	2
The hematoxylin methods	2
The acid anilin dye methods	2
The phosphomolybdic and phosphotungstic acid methods	2
The single solution methods	1
The elastic fiber methods	3
Stains for bacteria, fungi and inclusion bodies	7
Stains for cytoplasmic granules	4
Stains for hematologic elements	2
Stains for fats and lipids	3
Stains for carbohydrates and mucoproteins	6
Stains for pigments and minerals	1
Stains for nerve cells and fibers	3

Summary

The planning of a curriculum for teaching histologic technique to students of medical technology should follow a correlated pattern of classroom lecture and practical application in the laboratory. If the theory is omitted, the technic becomes a mechanical routine instead of an intelligent performance. If the practical application is omitted, the theory is meaningless, for the student does not have an opportunity to apply his knowledge. If we believe that the student of today is the chief technologist or teaching supervisor of tomorrow, our curriculum must be correlated, adequately prepared, carefully executed and practically applied, not only in histologic technic but in each laboratory division where students are trained.

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EXPERIENCES WITH THE PRECIPITATION TEST FOR LUPUS ERYTHEMATOSUS*

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Abstract

This paper deals with a series of lupus erythematosus precipitation tests and preparations performed on routine hospital admissions. The procedure used, results obtained and conclusions will be discussed.

Introduction

It is generally recognized that differentiation of disseminated lupus erythematosus from the other "collagen" diseases is often quite difficult. The demonstration of the distinctive, abnormal L.E. cell, first described by Hargrave, Richmond and Morton, is the most common criterion for the laboratory diagnosis of this disease. The L.E. preparation test is very time-consuming and requires careful examination by highly-skilled personnel. Since these personnel are not always available to physicians and time is always of the essence, technologists have had a very strong desire to find a simpler test for this disease.

In early 1958 Jones and Thompson presented an article describing a simple precipitation test for systemic lupus erythematosus. The authors claimed this test was unusually specific for L.E., giving false positive tests only in cases of multiple myeloma, viral hepatitis, disseminated coccidioidomycosis and granuloma of the bone. They stated that this test was negative in "collagen" diseases other than desseminated lupus erythematosus.

Discussion

Following the appearance of this article, we decided to run a series of L.E. precipitation tests to determine if this test could be used as an accurate screening test for lupus erythematosus. If the test proved to be specific and accurate, then smears would have to be made only on patients with positive precipitation tests. It was decided that we would use the serum collected for routine serologies on new admissions to the hospital for this testing.

The method used for this precipitation test required 0.1 ml. of serum, to which 2 ml. of 12% para-tolulene sulfonic acid in glacial acetic acid was slowly added. If there was precipitation or clumping the test was allowed to stand for thirty minutes. If, at the end of this period, the precipitate remained, despite agitation of the tube, the test was considered positive and a L.E. preparation was made. This method is identical with the method used by Jones and Thompson, the authors of the original article on this test. The method used for the preparation was: the clot from 5-10 ml. of blood was mashed through a fine wire sieve. The blood was then centrifuged for 5 minutes at 2000 rpm, the buffy coat removed and put in a Wintrobe hematocrit tube, centrifuged for 5 more minutes at 2000 rpm. Several smears were then made from the buffy coat in the Wintrobe tube. These smears were stained with

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Wright's stain and carefully examined for L.E. cells. On all of the positive precipitation tests, a Latex Fixation test for rheumatoid arthritis was performed to see if there was any correlation between these two tests. We felt this was indicated since the two diseases are so closely related. Since the precipitation tests were done on the serum collected for serologies, a VDRL was also done on each serum. It was noted that the serum which had been inactivated for the VDRL's would not yield as high a percentage of positive tests as fresh serum; therefore, we used only uninactivated serum for these tests. These precipitation tests were performed on approximately 70% of the patients admitted to the hospital during a 3½-month period. At the same time this series was being done, there were other L.E. precipitation tests done and L.E. preparations made at the request of physicians on specific patients. A very brief history was obtained from the charts of the patients with a positive L.E. precipitation test.

Results

The results of this series were:

L.E. precipitation tests done	1305
L.E. precipitation tests positive	25
Positive L.E. precipitation tests with positive R.A. tests	18
Positive L.E. precipitation tests with positive VDRL	2
Positive L.E. precipitation tests with positive smears	0

Smears done, at physicians request, on patients with negative precipitation tests during this time showed two positive smears with negative precipitation tests. Of these two, one of them had a positive precipitation test with a positive smear once and a negative precipitation test with a positive smear the following week. These were the only positive smears found in the laboratory during the time of this series.

The short history taken on the patients with positive precipitation tests gave us very little information. These patients ranged in age from 19 to 81 years. There were only three that were under 40. There were 14 females and 11 males. The diagnoses on these patients included cataracts, diabetic gangrene, cirrhosis, ureteral stones, appendicitis, hemorrhoids, cardiac conditions, etc. A few of the older patients had a history of arthritis and many of them were in the age group with which arthritis is most commonly associated. None of the patients had been diagnosed as lupus erythematosus or had any symptoms of the disease, with the exception of the two who had negative precipitation tests with positive smears.

Conclusion

Since we have finished this series, another article has been published on this test. Also there was a letter to the editor written regarding other work done along this line. Both of them were in the January 3, 1959 issue of the *Journal of the American Medical Association*. It seems that we had all reached the same conclusion. This being: this is a non-specific test, probably for an atypical globulin. Since 72% of the patients we tested that had a positive precipitation test also had a positive R.A. test, we came to the conclusion that there must be a

similarity between these reactions. Yet, since there were only 25 positives in the 1305 tests performed, we feel that many other patients with "collagen" diseases did not elicit a positive reaction. Others found a correlation between this test and the C-Reactive Protein phenomenon. Since it is so non-specific, we feel that it definitely cannot be used as a screening test for lupus erythematosus and that the L.E. preparation is still the best method for laboratory diagnosis of the disease. This precipitation is a very interesting phenomenon and we feel that further study of it is highly indicated. Since others have found it to be positive in large percentages of the patients with a diagnosis of disseminated lupus erythematosus, it might be well to use this test in conjunction with the preparation, but only as a method of adding a little more light to the subject—certainly not as a presumptive test for lupus erythematosus.

Summary

A series of 1305 L.E. precipitation tests performed on routine hospital admissions revealed that this test is not specific for disseminated lupus erythematosus and will not always be positive in proven cases of the disease. Therefore, we feel that it should not be used as a screening test for systemic lupus erythematosus.

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MODIFIED STAINING TECHNIQUE FOR BRAIN SECTIONS*

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Introduction:

The myelin stain previously used at this installation is one perfected by Colonel Hugh Mahon.[1] It is a modification of several methods using 1) the 4% iron alum of Weil's method[3] as a mordant and 2) the Borax solution of Weigert's method[2] as the differentiating solution. The method provides excellent blue to blue-black myelin staining against a nearly colorless background. Recently we have superimposed a Kernechtrot (nuclear fast red) counterstain on the method giving us excellent nuclear detail.

Method:

Fixation: 10% neutral buffered formalin
Technic: cut paraffin sections at from 6-18 microns

Solutions:

I.	Ferric Ammonium Sulfate Solution:	
	Ferric ammonium sulfate $Fe(NH_4)(SO_4)_2 \cdot 12H_2O$	4.0 grams
	Use only the lavender (fresh) crystals of ferric ammonium sulfate.	
	Distilled water	100.0 ml
II.	Stock Alcoholic Hematoxylin	
	Hematoxylin**	10.0 grams
	Absolute ethyl alcohol	100.0 ml
	(May be used immediately after preparation with no ripening period.)	
III.	Saturated Aqueous Lithium Carbonate.	
	Lithium Carbonate Li_2CO_3	1.54 grams
	Distilled water	100.0 ml
IV.	Dilute Lithium Carbonate:	
	Saturated aqueous lithium carbonate	7.0 ml
	Distilled water	93.0 ml
V.	Working Hematoxylin Solution:	
	Stock alcoholic hematoxylin (solution II)	10.0 ml
	Dilute lithium carbonate (solution IV)	90.0 ml
VI.	Borax Solution:	
	Potassium Ferricyanide $K_3Fe(CN)_6$	2.5 grams
	Borax (sodium borate) $Na_2B_4O_7 \cdot 10H_2O$	2.0 grams
	Distilled water	100.0 ml
VII.	Kernechtrot (Nuclear Fast Red) Solution:	
	Aluminum Sulfate $Al_2(SO_4)_3 \cdot 18H_2O$	5.0 grams

* Received for publication April, 1959.
** National Aniline, NA, 564.

Kernechtrot* ("chroma"—formerly Grübler stain)	0.1 gram
Distilled water	100.0 ml

Dissolve the aluminum sulfate with the aid of heat and while still hot "shower" in the dye. Cool, filter, add a grain of thymol as a preservative. May be re-used 4 or 5 times.

Staining Procedure:

1.	Xylene	
2.	Absolute alcohol	
3.	95% alcohol	
4.	Rinse in distilled water	
5.	Mordant in 4% iron alum (solution I)	30 minutes
6.	Rinse 2-3 times in distilled water (not over 30 seconds total)	
7.	Stain in working hematoxylin	60 minutes
8.	Wash in distilled water	
9.	Blue in dilute lithium carbonate (solution IV)	30 seconds
10.	Wash in distilled water.	
11.	Differentiate in Borax solution	30 seconds
12.	Wash in distilled water.	
13.	Counterstain with Kernechtrot.	5 minutes
14.	Wash well in distilled water.	
15.	95% alcohol.	
16.	Absolute alcohol.	
17.	Carbol-xylol.	
18.	Xylene.	
19.	Mount in permount.	

Results:

Myelin	Blue to blue black
Red blood cells:	Black
Nuclei:	Red
Nuclei of neurons:	Dark red
Cytoplasm:	Pink
Background	Pale pink

Discussion

This staining method more readily demonstrates the association of the fibers of the cranial nerves with their nuclei (Fig. 1) than does either staining by hematoxylin and eosin or methods for demonstrating myelin sheaths. The adequacy of the stain may be ascertained by noting the color of the red blood cells. In all slides we have studied, if the red blood cells are black, any myelin present will be stained the characteristic blue to blue-black.

Summary

A method for superimposing a Kernechtrot (nuclear fast red) counterstain on a myelin sheath stain is described. This combination gives a sharp nuclear detail as well as a myelin stain.

* May be obtained from Roboz Surgical Instrument Company, Washington 7, D. C.

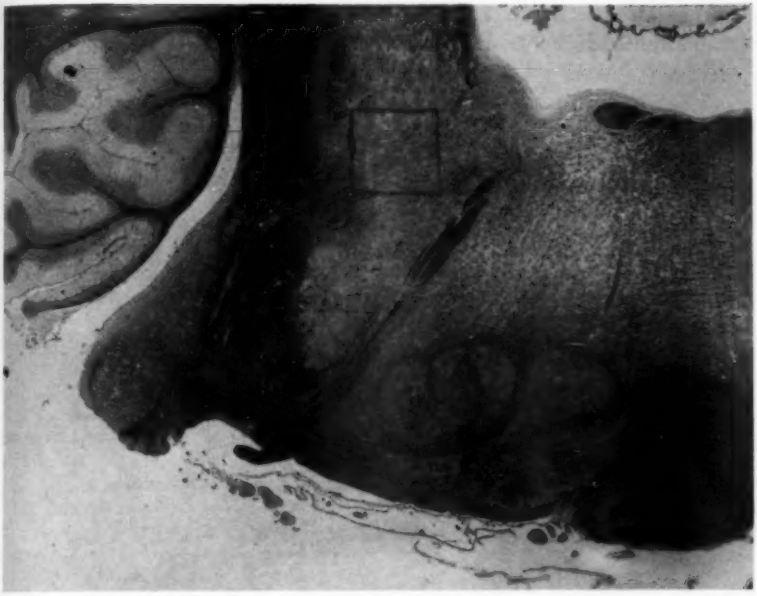

Figure 1
(MRNL 868) Cross section of medulla oblongata, just posterior to the Pons, Canine. Nuclear Fast Red-Myelin Stain, 48 mm.

Figure 2
(MRNL 868) Portion of Figure 1, outlined. Nuclear Fast Red-Myelin Stain, 90X.

Acknowledgment

All photomicrographs were taken by Mr. William Hummel of the Pathology Service, Fitzsimons Army Hospital, Denver 30, Colorado.

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CORRECTION

Due to an unfortunate set of circumstances, "Check List of Scientific Journals and Allied Periodicals for the Clinical Laboratory," by John S. Hannan, MT (ASCP), which appeared in the May-June issue of this journal did not include two publications:

Lab World Monthly news magazine of the clinical-scientific laboratory field. In addition to news stories, contains departments of Dates to Remember, Books, Research Briefs, New Aids, and Journal-Ease. The last-named is a listing of titles of recently published journal articles with the name and address of the author(s) and the journal in which it may be found. These titles are classified under the headings of Biochemistry; Biochemistry, Special Instrumentation; Equipment; Hematology; Histopathology; Microbiology; Physiology; Radioisotopes; Serology; Virology; Rickettsiology; Miscellaneous; and X-Ray. Some of the listings include a very short outline of the contents of the article.

Labstracts A companion publication to the above, published yearly, consisting of some 1800 references compiled from the preceding year's issues of *Lab World*. Other sections are an extensive source list of journals, a list of organizations and associations, and a source list of films.

The address of the office of publication for both is 672 S. Lafayette Park Place, Los Angeles 57, California.

A RAPID, RELIABLE PROCEDURE FOR DETERMINATION OF SERUM PROTEIN-BOUND IODINE*

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The determination of serum protein-bound iodine is generally considered to be one of the more difficult analytical procedures with which the hospital laboratory is confronted, principally because micro quantities are involved and the ever present possibility of contamination which can lead to erroneous results. The chloric acid method for determining protein-bound iodine is less hazardous than some of the analytical procedures performed routinely in the clinical laboratory, provided certain precautions are observed, and is apparently the method that fulfills the need for a simple, rapid, and reliable procedure which the medical technologist can perform with minimum difficulty. This method is comparatively rapid, because more than 20 specimens can be processed in triplicate analysis in one day. The advantage of triplicate analysis of each specimen eliminates any doubt concerning the validity of results, particularly in a procedure, such as this, which deals with the estimation of microgram quantities.

The method under discussion is essentially a modification of the chloric acid procedure originally proposed by Zak[1] and later revised by others[2,3,4]. The procedure used by the authors has proved to be unquestionably satisfactory in several hundred determinations, during a period of one year, with an accuracy within 0.5 microgram compared with the methods of Barker and Chaney (Table 1). The success of the chloric acid method for accurate determinations of serum protein-bound iodine is governed by the following factors: (1) the water used in the reagents and in the procedure proper must be triple-distilled and stored in Pyrex or polyethylene containers; water purified with research model ion-exchange de-ionizers is very satisfactory and recommended. (2) The temperature during the digestion process must be properly controlled to preclude loss of iodine; this is the most critical step in the procedure. (3) Consistently reproducible results are obtained when the aqueous iodine standard is added to reference control serum to duplicate as nearly as possible the composition of the unknown serum specimens carried through the analysis. (4) The procedure need not be carried

TABLE 1

Comparison of Results of Typical Analyses of Protein-bound Iodine by the Proposed Method with Those of the Methods of Barker and Chaney

Sample No.	Proposed Method	Barker Method	Chaney Method
1	4.8 ug./100 ml.	4.4	4.7
2	5.0	5.3	5.1
3	9.7	10.1	10.4
4	4.5	4.4	4.6
5	4.9	4.7	5.1
6	3.9	4.3	4.1
7	3.2	3.1	3.6

*Received for publication April 1959.

out in a separate room from the laboratory, but at least fifteen feet away from iodine-containing reagents, such as Nessler's reagent. Separate equipment and glassware are absolutely necessary.

PRINCIPLE

Iodine in protein-precipitated serum is oxidized to the iodate form during digestion with approximately 28 per cent chloric acid in the presence of chromate. The iodate will not volatilize with the chloric acid, provided the temperature is carefully controlled. Following digestion, the iodate is reduced to the iodide state by the addition of arsenious acid. A critical amount of sodium chloride must be added with the arsenious acid to accelerate the final reaction. In the final reaction, the iodide catalyzes the reduction reaction of the cerium-arsenite system, following the addition of ceric ammonium sulfate. Iodide acts as a catalyst in the following reaction:

$$2\,Ce^{++++} + H_2A_sO_3^- + H_2O \rightarrow 2\,Ce^{+++} + H_2A_sO_4^- + 2H^+$$

Yellow cerium ion	Colorless (reduced) cerium ion

In the above reaction, the extent of reduction of the cerium ion is proportional to the quantity of iodine in a serum specimen.

REAGENTS AND MATERIALS

All stock reagents should be of the purest grade available. Volumetric flasks with ground-glass stoppers should be used.

1. **15 per cent trichloroacetic acid,** weight-volume.
2. **0.5 per cent sodium chromate,** weight-volume.
3. **28 per cent chloric acid.** Rubber gloves should be worn, and the solution prepared in a fume hood with the door of the hood partially closed. In a 2-liter Pyrex Erlenmeyer flask with ground-glass stopper, add 540 ml. of triple-distilled (de-ionized) water to 300 grams of potassium chlorate crystals. Heat the mixture on an electric hot plate until the crystals are completely dissolved. To the syrupy solution, while still near the boiling point, add 250 ml. of perchloric acid (70-72%) in small portions with rapid swirling of the flask. Any spillage of perchloric acid should be cleaned promptly with copious amounts of water. Allow the solution to cool to room temperature in the fume hood before placing the stoppered flask in the refrigerator overnight. Next day, filter the cold supernatant liquid into a polyethylene bottle through Pyrex glass wool or Whatman #40 filter paper. Approximately 500 ml. of light green filtrate should be obtained. Store in the refrigerator. Promptly wash the residue in the Erlenmeyer flask down the drain with cold water, since this residue is explosive in the dry state.
4. **0.2 normal arsenious acid.** Dissolve 9.8910 grams of arsenic trioxide and 7 grams of sodium hydroxide in 100 ml. of triple distilled water in a liter volumetric flask. Dilute to about 400 ml. with water and neutralize to phenolphathalein with concentrated sulfuric acid (takes 4-5 ml.). Add 42 ml. of concentrated sulfuric acid and dilute to 1 liter with water. The final acidity is approximately 1.5 normal.

Store in a polyethylene bottle in a dark place at room temperature.

5. **0.1 normal ceric ammonium sulfate.** Use ceric sulfate manufactured by the G. Frederick Smith Chemical Company, Columbus, Ohio. This solution is prepared in two steps:

   A. **Step #1.** Prepare 250 ml. of 0.5 normal ceric sulfate by placing 25 grams of the salt in a 250-ml. volumetric flask. Add 15-16 ml. of concentrated sulfuric acid. Swirl the flask vigorously to dissolve as much of the ceric sulfate as possible. Slowly add water in small volumes while swirling the flask vigorously. Dilute to volume with water, and invert the flask several times to completely dissolve the ceric sulfate.

   B. **Step #2.** Transfer 200 ml. of the 0.5 normal ceric sulfate solution to a liter volumetric flask. Dilute to about 600 ml with water. Add 13.2 grams of ammonium sulfate. Mix. Add 83 ml. of concentrated sulfuric acid. Mix. Add 2 grams of sodium chloride crystals. Dilute to volume with water. The final acidity is approximately 3.2 normal. Store the solution in a polyethylene bottle in a dark place at room temperature.

6. **2 per cent sodium chloride solution,** weight-volume.

7. **Stock iodine standard solution** (100 mcg./ ml.). Weigh accurately with an analytical balance 0.1685 gram of desiccated potassium iodate, KIO_3, and dilute to 1 liter with water. Invert the flask about 100 times for complete mixing before storing the solution in a polyethylene bottle under refrigeration.

8. **Iodine working standard** (0.1 mcg./ml.). Dilute 1 ml. of the stock standard solution to 1 liter. Mix thoroughly. Prepare this solution on the day prior to the day of analysis, for complete mixing. This solution can be used for one week.

9. Commercially available normal and abnormal lyophilized control serum with known concentrations of protein-bound iodine.

APPARATUS

1. 16 Glass Beads, solid, 3 mm. diameter.

2. Heavy duty Pyrex centrifuge tubes with pouring lip, 40-ml. capacity; and 4 wire test tube supports, 13x5½ inches with 30-mm. spaces, suitable to each hold 40 of the centrifuge tubes vertical.

3. Sand-bath. An aluminum pan 12x14x4½ inches filled with a 1 to 2 inch layer of purified sea sand is suitable. This pan is easily constructed without the use of rivets by folding a 25x21 inch sheet of aluminum cut with sheet-metal shears. Aluminum sheets 36x36 inches are available at local hardware stores. Two of the 40-place centrifuge tube supports are sunk in the sand in the aluminum pan.

4. Temco electric hot plate 12x12 inches with a 5-inch wide aluminum shield attached around the top of the hot plate to surround and retain heat in the sand-bath. The 12x14 inch aluminum pan extends over one side of the hot plate, but this does not interfere with heat distribution in the sand. The aluminum shield is tucked under the extended side of the aluminum pan resting on top of the hot plate.

5. Pyrex test tubes, 18x150 mm., and a constant-temperature water-bath large enough to accommodate 1 or 2 racks to hold the test tubes vertical. An enamel ware tray 16½x12¼x2½ inches placed over a 6-inch Fisher autemp heater is satisfactory.

PROCEDURE

A. Protein Precipitation

Transfer exactly 1 ml. of freshly reconstituted normal control serum, with a known value near 5 mcg./100 ml., into each of 4 duplicate 40-ml. heavy duty Pyrex centrifuge tubes marked S_1, S_2, S_3, and S_4 with a carbon pencil. Transfer 1 ml. of abnormal control serum and 1 ml. of unknown serum into similar, properly marked, triplicate sets of centrifuge tubes.

Draw 15 per cent trichloroacetic acid solution into a 10-ml. volumetric pipette. Slowly add about half of the acid solution to each tube while briskly swirling the tube during the addition for complete precipitation of serum protein. Blow the remainder of the acid solution against the walls of the tube to wash down any precipitate clinging to the sides. Save the pipette for further addition of acid solution.

Centrifuge all tubes at 3000 r.p.m. for 5 minutes, then decant the clear supernatant liquid into a sink. This supernatant liquid contains inorganic iodine, and it may be included along with the analysis of the serum protein-bound iodine, if desired.

Add with strong pressure an additional 10-ml. volume of 15 per cent trichloroacetic acid solution against the tightly-packed precipitate in the bottom of each tube to disperse the precipitate. Wash down precipitate clinging to the walls of the tubes. Recentrifuge for 5 minutes at 3000 r.p.m. Discard the supernatant liquid.

Invert each tube over 2 or 3 layers of large filter paper sheets resting on the middle shelf of a wire rack for a period of 3 to 5 minutes for complete drainage of trichloroacetic acid solution.

The precipitation of serum protein as outlined above may be done near the end of the day prior to the day of analysis, to save time. The tubes may be kept inverted and should be placed in the refrigerator overnight.

Prepare the 0.1 mcg./ml. iodine standard and allow it to stand overnight for complete mixing. Estimate the volume of 28 per cent chloric acid needed (10 ml. per tube) and allow it to stand at room temperature overnight, because refrigerated chloric acid produces considerable "bumping" in the centrifuge tubes at the beginning of the digestion.

B. Preparation for Digestion

Place 1 ml. of triple-distilled or de-ionized water in a properly labeled 40-ml. Pyrex centrifuge tube for the reagent blank.

Add 0.5 ml., 1 ml., and 1.5 ml. of the aqueous 0.1 mcg./ml. iodine standard to the centrifugates in the tubes labeled S_2, S_3, and S_4, respectively. This represents an addition of 5, 10, and 15 micrograms of iodine per 100 ml. and gives standards with values near 10, 15, and 20 micrograms of iodine per 100 ml. The tubes marked "S_1" contain the precipitated control serum with a known value near 5 mcg./100 ml. and serve the purpose of the 5 microgram standard for the calibration curve in the colorimetric analysis.

Add 6 glass beads to each tube to prevent or minimize "bumping" during the digestion.

Add exactly 1 ml. of 0.5 per cent sodium chromate solution to all the tubes.

Add 10 ml. of 28 per cent chloric acid which has been brought to room temperature. Use a suction bulb to draw the acid solution into a graduated pipette, and force the drainage of the pipette to loosen the centrifugates in the bottom of the tubes. Mix well, but avoid getting precipitate on the walls of the tubes.

C. **Digestion**

The digestion must be carried out in a fume hood. It is advisable to have the door of the hood open about one inch at the bottom to prevent unequal air flow and to protect the laboratory personnel against inhaling the perchloric acid fumes. It is also advisable to wipe the working surface and the lower walls of the hood with a damp sponge once a week to minimize the accumulation of dust and perchloric acid sediment.

Place all tubes in a strictly vertical position in the sand-bath. Press each tube to a depth of about ¼ to ½ inch in the sand. Turn on the hot plate.

Place in the sand-bath a 40-ml. Pyrex centrifuge tube filled with about 20 ml. of distilled water, preferably in the hottest area of the sand-bath; and place a 110° C. thermometer in the water. Do not allow the temperature of the water to exceed 89° C. until the precipitates in all the tubes are dissolved, otherwise iodine might be lost due to rapid initial heating.

Allow the liquid mixtures to evaporate at a constant temperature maintained between 88 and 90° C. registered by the centrifuge tube with the water. Vigorous, rapid boiling must be avoided. Tubes boiling vigorously should promptly be raised out of the hot sand or moved to a cooler area in the sand-bath. The yellow color of the solutions gradually changes to orange-red and the solutions acquire a syrupy appearance near the completion of the digestion. A green color should not develop once the precipitates are all dissolved, otherwise iodine might be lost due to gross reduction of chromate. This should not occur if the temperature of the tube of water is not allowed to exceed 90° C. at any time during the digestion process.

When the volume of the solutions is reduced to about 1.5 ml., the tubes must be closely watched. The end of the digestion is reached when the chromic acid precipitates and red crystals begin to appear on the surface of the solutions around the walls of the tubes, about 3 hours from the beginning of the digestion. Promptly remove from the sand-bath the tubes containing red chromic acid crystals and allow the tubes to cool to room temperature in the fume hood, away from dust or fumes. The volume of the solution mixtures is invariably 1 ml. when chromic acid crystals appear, indicating that the reaction is completed.

D. **Preparation for Colorimetric Assay**

Add exactly 9 ml. of triple-distilled or de-ionized water to each tube, from a 50- or 100-ml. burette with a side tube for refilling from a

reservoir. Place the rack of tubes over the sand-bath with the hot plate turned low, to hasten solution of the particles of soluble salts. The particles dissolve completely in about 10 minutes, when the tubes are warmed to about 45° C.

Adjust the constant-temperature water-bath at 30° C. Transfer exactly 3 ml. of the clear solutions from the digestion tubes (cooled to room temperature) to 150x18 mm. Pyrex test tubes. Add exactly 2 ml. of 0.2 normal arsenious acid reagent and mix by lateral rotation of the tubes. Then add 0.5 ml. of 2 per cent sodium chloride solution to each tube. Mix thoroughly by lateral rotation. Place the test tubes in the water-bath at 30° C. for 20 minutes.

E. Colorimetry

Since colorimetric readings are usually made in 20-30 minutes, the maximum number of tubes that can be processed at one time in this step should be limited to 40, unless an additional technologist is available to begin the colorimetric readings at the appropriate time while the color reagent is being added to the tubes in excess of 40.

With the test tubes kept in the water-bath at 30° C., add to each 1 ml. of the ceric ammonium sulfate reagent at accurately timed intervals of 30 seconds, using a stopwatch. Mix well by lateral rotation as soon as the reagent is added. Set a time clock at 20 minutes as soon as the ceric ammonium sulfate reagent enters the first tube. The 150x18 mm. test tubes used by the authors are calibrated for the Beckman DU Spectrophotometer equipped with a test-tube adapter. If the solutions must be transferred to cuvets, it is advisable to allow the reaction to proceed to completion in the large test tubes before the transfer. The photometer or colorimeter is adjusted at zero absorbance, or 100 per cent transmittance, with triple-distilled or de-ionized water at 420 millimicron wavelength (blue filter).

At the end of 10 to 15 minutes, after the addition of the ceric ammonium sulfate, the optical density or transmittance of the reagent blank, of the 5-mcg. standard (S_1), and of the 20-microgram standard (tube S_4) should be read at frequent intervals. As soon as the blank attains an optical density reading between 0.75 and 0.70 (18 and 20 per cent transmittance), the optical density reading of the 5-mcg. standard is between 0.60 and 0.56 (25 and 28 per cent transmittance), and the optical density reading of the 20-mcg. standard is between 0.25 and 0.20 (56 and 63 per cent transmittance), the final readings of all the solutions should be started. Readings of each tube are made at 30-second intervals, using a stopwatch, and in the same order as the addition of the ceric ammonium sulfate reagent.

F. Calculations

A new calibration curve must be constructed at the end of each analysis, by plotting the optical density readings of the four standards against their concentrations on linear paper ruled 10x10 to the centimeter. If the transmittance is read, plot on semi-logarithmic paper. The curves are straight lines, but do not necessarily go through the origin. From the calibration curve, the concentrations of the control serum and of the unknowns are obtained. The reagent blank, assuming

it is not contaminated with extraneous iodine, usually contains 0 to 0.2 microgram of iodine per 100 ml. The concentration of iodine in the reagent blank, if any, should be subtracted from the concentration of iodine found in the unknowns (Fig. 1).

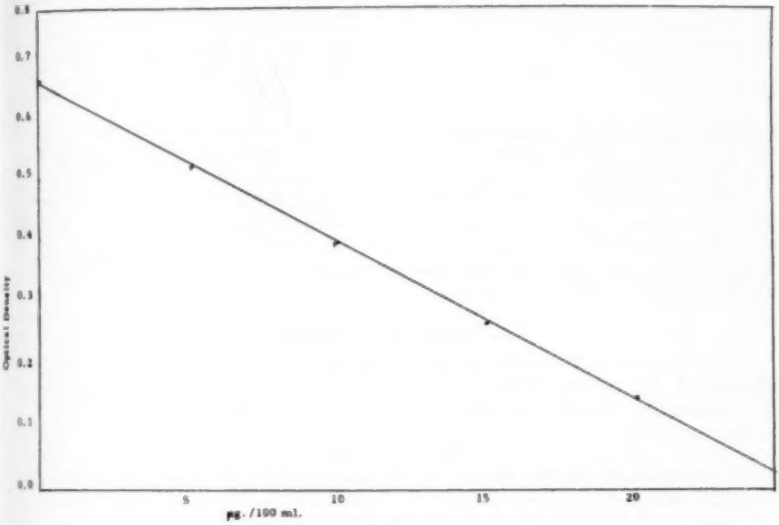

Figure 1
A typical calibration curve obtained in a daily analysis

G. Cleaning of Glassware

The pipettes, test tubes, centrifuge tubes, and glass beads are most satisfactorily cleaned by allowing them to remain completely submerged in potassium dichromate-sulfuric acid solution for at least 12 hours. All traces of chromate should be removed from the centrifuge tubes used for the digestion; this is accomplished by rinsing them individually with 25-50 per cent sodium or potassium hydroxide solution. Likewise, all traces of hydroxide must be removed by thorough rinsing with water. Rinse all tubes under running warm tap water at least 5 times and 3 times with triple-distilled or de-ionized water. The pipettes should be rinsed at least 5 times in a pipette rinser, followed by rinsing with distilled water.

SUMMARY

A simplified, accurate, and dependable procedure for the determination of serum protein-bound iodine is described in detail. Any standard photoelectric colorimeter or spectrophotometer may be used to attain reproducible results (Table 2). The use of human serum reference standards of known iodine concentrations that duplicate the physical similarities and chemical reaction of serum from the patient, fortified with known concentrations of an accurate aqueous iodine standard,

TABLE 2
Recovery of Iodine Added to Control Serum Samples

Sample No.	μg./100 ml.	Iodine Added As KIO_3	μg./100 ml. Iodine Recovered
1	5.2	5.0	10.3
2	4.4	5.0	9.4
3	4.4	10.0	14.5
4	5.1	15.0	20.3
5	5.1	15.0	20.1

produces results of the unknowns which can be compared to the plotted curve of the standards for consistently accurate protein-bound iodine estimations. Triplicate analyses of each unknown, coupled with the simultaneous analyses of normal and pathological serum controls with known values, provide multiple checks of the final results. It is hoped that the detailed description of the procedure should enable medical technologists to perform the analysis without the necessity of further major modification.

ACKNOWLEDGMENT

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ISOLATION AND CHARACTERIZATION OF AN UNUSUAL ORGANISM IN BLOOD CULTURE: *VIBRIO FETUS**

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The difficulties encountered while establishing the identity of an organism found in a human blood culture as *Vibrio fetus* prompted the preparation of this review. It is hoped this report will be of help to others who have a similar problem.

Many reports have been published in connection with the serological and cultural identification of *Vibrio fetus* of bovine origin.[2,5,6,9,11,12,15] For years infections with this organism were associated with animals, and only recently have instances of vibriosis been reported in human beings.[7,16,17]

The present study relates the characteristics of *Vibrio fetus* and the methods used for its identification. Because of the repeated recovery of the organism from routine blood culture, a short history of the patient is included.

CASE REPORT

A 68 year old white, male farmer was admitted to the University Medical Center three times with a history of fever and chills. The patient had contact with the usual farm animals, and had been bitten frequently by ticks. Dead rats had been observed on the farm. Past history was negative for serious illnesses, except for scarlet fever as a child. The febrile agglutinations including agglutination for *Brucella abortus* were negative. On the first admission a spiral organism was cultured from blood on three occasions. Two months later the patient was readmitted with the same symptoms, and two more positive blood cultures were obtained. After treatment with chlortetracycline the patient was dismissed with no further symptoms of septicemia.

MATERIALS AND METHODS

Biochemical and cultural media: The blood culture media consisted of brain heart infusion broth with the addition of 0.1 per cent agar and 0.02 molar magnesium sulfate. Difco purple base broth with one per cent of the fermentable substance (lactose, dextrose, sucrose, maltose, d-xylose, d-mannitol, and salicin) was used for carbohydrate fermentation tests. All cultures were incubated in approximately 3.5 per cent carbon dioxide in a candle jar and also aerobically at 37° C. Difco blood agar base with 5 per cent human blood was used for isolation plates. Chocolate agar plates were made from blood agar base with hemolyzed human blood. The catalase test was performed by adding approximately 1 ml. of 3 per cent hydrogen peroxide to a 96 hour culture on trypticase soy agar slant, and effervescence was observed. The oxidase test was performed by adding a drop of a one per cent solution of tetramethyl-p-phenylene-diamine dihydrochloride to a 96 hour growth on blood agar plate, and the changing of the colonies to a

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black color was noted. Kligler iron agar was used for production of hydrogen sulfide. Motility was demonstrated in hanging drop preparations and in semisolid media. All slants, plates, and broths were inoculated with loop from a 96 hour culture on blood agar.

Maintenance of stock cultures: Cultures were maintained by weekly transfers in brain heart infusion broth under carbon dioxide in candle jar at 37° C. The organisms were preserved by freezing on beads. The beads, placed in a glass tube, were inoculated dropwise with a 96 hour broth culture of the organism. A desiccant was placed in the tube, and the inoculated beads were dried *in vacuo* at room temperature. The tube, sealed off by heat, was stored at —10° C.

Serums: Serums, H5-30-58, H7-01-58, and H7-29-58, were collected from the patient with vibriosis. The initial represents the patient's name, and the numbers indicate the dates when the serums were drawn. RAB I and RAB II are the serums collected from two rabbits immunized against the organism. Ten serums collected at random from hospital patients were used for controls.

Preparation of Antigen. Bottles of brain heart infusion broth were inoculated with a loop from a 96 hour growth on blood agar plate. The bottles were incubated in a candle jar at 37° C. for 96 hours. After centrifugation at approximately 8,000 r.p.m. in a Servall Superspeed Centrifuge for 10 minutes and decantation of the supernatant, the organisms were harvested in an approximately equal volume of 0.5 per cent phenolized saline.

Preparation of Antiserums: The antigen, adjusted to the turbidity of a No. 2 McFarland Standard, was given intravenously to two adult rabbits. The six injections, (0.5 cc., 1 cc., 2 cc., 3 cc., 3 cc., 3 cc.) were given at 4-5 day intervals. The animals were bled from the heart two weeks after the last injection and the serum preserved at 4° C.

Agglutination test: Antigen was prepared in manner described for immunization of rabbits. Serial dilutions of antiserums and serums were prepared in saline ranging from 1:10-1:5120; and 0.5 ml. amounts were placed in serological tubes. An equal amount of antigen was added to each; and the tubes were then incubated in a water bath at 52° C. for two hours followed by overnight incubation at 4-6° C. The highest final dilution showing a 2+ reaction, agglutination with incomplete clearing of supernatant, was taken as titer.

Complement fixation test: Antigen was prepared in the same manner as described in the agglutination test. The Fifty Percent Complement Fixation Method of the Serology Research Laboratory, Communicable Disease Center, Chamblee, Georgia, was followed throughout. In this method the highest final dilution giving a 50 per cent hemolysis, determined by color standard, was taken as the titer.

RESULTS

Cultural Studies: The blood cultures were considered positive when small puff ball shaped colonies were seen between the blood cells and the supernatant. The organisms from all blood cultures were alike morphologically, appearing as small, slender, curved, gram negative rods with many spiral forms. The organisms, approximately 0.2-0.5 by

1.5-5.0 microns, stained well with ordinary anilin dyes, crystal violet giving the best results (Figure 1). The hanging drop preparation under the phase microscope showed the organisms to be very active, moving in a twisting motion across the field.

The organisms grew on blood agar and in brain heart infusion broth under reduced oxygen tension, but very slowly and poorly under atmospheric conditions. The candle jar proved to be most satisfactory for both solid and liquid media. On human blood agar plates, a slight growth was noted in 24 hours. After 72 hours, the colonies were gray, opaque, glistening, smooth, flat, and round (Figure 2). Upon transfer to brain heart infusion broth the growth first appeared cloudly throughout, but after 72 hours settled to the bottom as a stringy, viscid, mucoid mass.

The organisms were catalase and oxidase positive. No growth occurred on Simmon's citrate agar or in urea broth. Carbohydrates were not attacked, indol was not produced, and hydrogen sulfide was not produced on Kligler iron agar. There was no growth on Salmonella-Shigella agar, or chocolate agar using G. C. medium base, however, when blood agar base was substituted for the G. C. medium base in the preparation of chocolate agar plates, the organism grew equally as well as with blood agar plates. The same amount of growth was obtained on a plate of blood agar base without blood. Trypticase soy agar, with or without blood, used in place of blood agar base gave the same results.

Agglutination tests: The three serums of the patient (H5-30-58, H7-01-58, and H7-29-58) and the rabbit antiserums (RAB I and RAB II) had high titers of agglutinating antibody when tested against homologous antigens (Table 1). The control serums gave no evidence of antibody production.

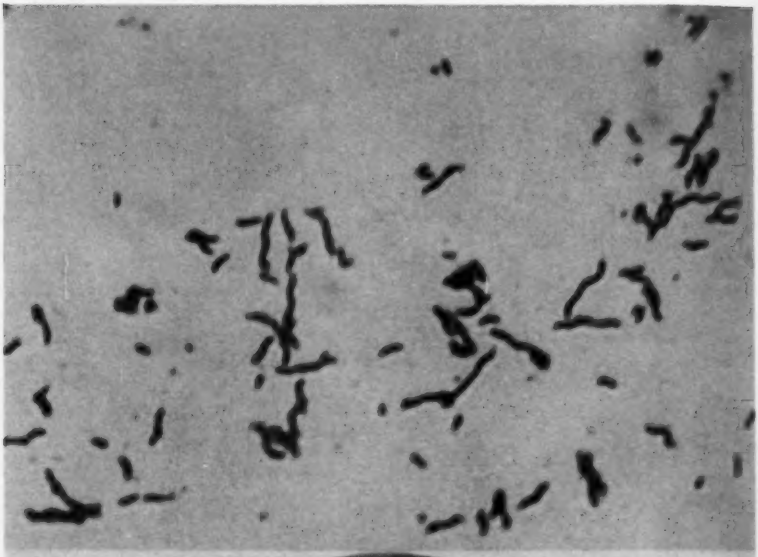
Complement fixation tests: Using a 1:4 dilution of the antigen, high titers of complement fixing antibodies were found in the three serums from the patient with vibriosis and in the serums from the immunized rabbits (Table 1). The negative results of the control serums indicated no complement fixing antibodies against *V. fetus* and no cross reacting antibody.

TABLE 1
Results of Serological Tests

TESTS	SERUMS					
	H5-30-58	H7-01-58	H7-29-58	RAB I	RAB II	Control
Macroscopic Agglutination.....	1:1280	1:1280	1:1280	1:1280	1:1280	Neg.
50% Complement Fixation..	1:256	1:128	1:256	1:256	1:256	Neg.

Animal virulence tests: Intravenous and intraperitoneal injections of 1 cc. of a 72 hour culture of the organism into rabbits did not produce any evidence of clinical infection. Mice, guinea pigs, and hamsters were also given intraperitoneal inoculations of 0.1 cc., 0.5 cc., and 1 cc. of a 72 hour culture. The animals were killed at intervals of one to six

Figure 1
Typical appearance of the cultivated *V. fetus* stained with crystal violet, x 3000



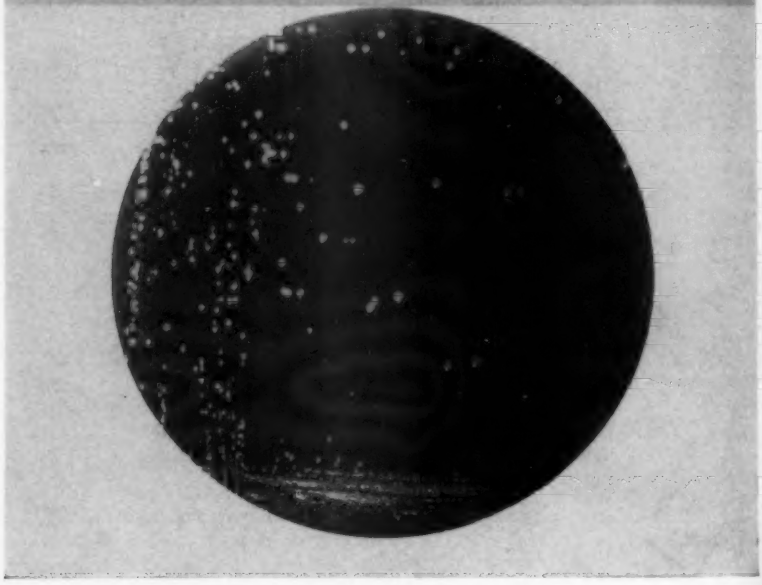


Figure 2
Vibrio fetus colonies on blood agar plate after 72 hours incubation in candle jar at 37°C

weeks after inoculation, and no organisms could be demonstrated either by smear or from the cultures of kidney, spleen, heart, and liver obtained at autopsy.

DISCUSSION

According to Bergey's Manual of Determinative Bacteriology the organism recovered belonged to the family *Spirillaceace.*[1] It was unlikely that it should be in Genus VII, *Spirillum,* since the only species recognized as pathogenic for man was *Spirillum minus. Spirillum minus* has not been grown on artificial media; however, Hetzig and Leibesman reported that a *S. minus* was recovered from blood culture of a case of subacute bacterial endocarditis.[4] The cultural, biochemical, and morphological characteristics led to the belief that this organism must be in Genus I, *Vibrio.* The production of catalase and the absence of hydrogen sulfide production coincided with growth characteristics described by Bryner and Frank for the identification of *V. fetus.*[2] A subculture of this isolate sent to Miss Elizabeth King of the Bacteriology Diagnostic Laboratory of the Communicable Disease Center, Chamblee, Georgia, was identified as *V. fetus.* According to the cultural and biochemical characteristics of the spirillum described by Lamb and Paton,[10] and Shwartzman et. al.,[14] their organisms were similar to King's[6] reported observations of *V. fetus.* The results and observations in my work, with the same results by King, suggested that their organisms probably were *V. fetus* (Table 2).

Also recorded in Table 2 are results obtained by others who have reported spiral organisms. The findings of Spink[16] and Ward,[17] even though of limited scope, indicated the organism was *V. fetus.* The fact that anaerobic growth was observed by Curtis[3] indicated that he recovered a different organism.

Serologically, this work showed that high titers were obtained from both agglutination and complement fixation tests. Also, serological studies of this organism carried out at the Communicable Disease Center resulted in high titers with antiserums prepared against other human strains of *V. fetus.*[8] An interesting fact was revealed by the complement fixation test. The second specimen of serum, H7-01-58, taken while the patient was convalescing revealed a drop in antibody titer from the first serum, H5-30-58. However, when the third serum, H7-29-58, was collected, the patient was having a recurrence of the disease. The rise in titer in this last serum indicated that antibody response occurred only when the patient had an active infection. This fact was not evident in the agglutination results. Unfortunately, since the patient was dismissed, a later serum could not be obtained to further support this theory.

Serums obtained from another patient with a similar illness revealed low titers of agglutinating antibody; whereas, complement fixation tests were anticomplementary. The ten control serums gave no evidence of antibody titer.

It is believed that this organism may be more prevalent in human infections than the number reported. Laboratory personnel should bear in mind that diseases resembling brucellosis, and possibly undiagnosed cases of fever may be due to this unusual organism. Agglutination and

TABLE 2

Comparison of Biochemical and Cultural Results of Reported Spiral Organisms

BIOCHEMICAL & CULTURAL MEDIA	AUTHORS							
	Spink[16]	Ward[17]	Curtis[3]	Hitzig[4] & Leibeaman	Lamb[10] & Paton	Shwartzman[14]	King[7,8]	This Paper
Blood Agar	....	....	+	—	+	+	+	+
Plain Agar	....	....	—	—	+	+	+	+
Chocolate Agar	....	....	..	—	..	+	..	+
Maconkey Agar	....	....	..	.....	..	..	+	+
Glucose Agar	....	....	+	.....	+	..	..	..
Gelatin	....	—	—	—	—	..	—	—
Albimi Brucella Agar	+	....	..	.....	..	..	..	..
Trypticase Soy Broth	+	....	..	.....	..	..	..	++
Thioglycollate Broth	..	+	..	.....	..	..	+	..
Veal Infusion Broth	....	....	..	+	..	..	..	..
Tomato Extract Broth	....	....	..	+	..	..	..	..
Dextrose Broth	....	....	..	+2%	+	..	..	..
Lactose Broth	....	....	..	..	++	..	..	..
Plain Broth	....	....	..	.....	..	..	..	..
Brain Heart Infusion Broth	....	....	..	.....	..	..	..	+
Tryptose Phosphate Broth	+	....	..	.....	..	..	..	..
Loeffler's Serum Slant	....	....	..	.....	+	..	..	+
Litmus Milk	....	....	—	.....	—	..	..	—
Oxidase Production	....	....	..	.....	..	..	..	+++
Catalase Production	....	....	..	.....	..	..	++	+++
Nitrate Reduction	....	....	..	.....	..	—	—	—
Indol Production	....	....	..	.....	..	—	—	—
Citrate, Urea, MR/VP	....	....	..	.....	..	—	—	—
Hydrogen Sulfide Production	....	....	..	.....	..	—	—	—
Carbohydrate Fementation	....	—	..	.....	—	—	—	—
Carbon Dioxide Requirement	10%	10%	..	3.5%*	..	+	3.5%*	3.5%*
Anaerobic Growth	....	....	+	.....	facultative	..	..	..
Aerobic Growth	....	....	..	.....	..	+	..	..

* Candle jar.

Blank spaces indicate no results reported.

complement fixation studies might provide useful epidemiological data in the rapid diagnosis of infection. Although skin testing was not used in this study, the possibility of it as a diagnostic tool should not be overlooked.

SUMMARY

A spiral organism was repeatedly recovered by blood culture from a patient with fever and chills. Morphologically and biochemically it resembled *Vibrio fetus,* an organism causing abortion in cattle. Identification serologically proved it to be the same. Immunological study revealed a rise and fall of complement fixing antibody titer within the patient's serum. Little or no cross reaction was observed with the serums collected from a patient with a similar infection, or with control serums.

Comparison with other published reports of similar organisms isolated from human beings suggests that this organism is being overlooked. It is possible that *Vibrio fetus* is more common in human infections than realized.

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STANDARD DEVIATION AS APPLIED TO HEMATOLOGY†

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Even under optimal working conditions there is an inherent variability in hematology as it deals with biologic material.[4] Statistical methods allow techniques to be studied in order to estimate their degree of accuracy. The purpose of this paper is to acquaint readers with a statistical method of evaluating major hematological procedures.

Population in a statistical sense consists of all members of a group having certain distinguishing characteristics in common. If it were possible to take measurements of some characteristic of all individuals in a population, values would vary from individual to individual. Classifying these values as to frequency of occurrence and plotting these frequencies against the deviation of the values from the mean or average, one would obtain a normal frequency distribution curve.[1] (Figure 1).

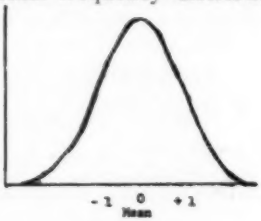

Figure 1
Normal Frequency Distribution Curve

Using this same technique one can measure the reproducibility of a test by performing multiple determinations on a single specimen. A high narrow distribution curve indicates that there is little deviation of most values from the mean. A low flat curve indicates that most values vary widely from the mean and, therefore there is a large error under the conditions of the test.

Calculation of the standard deviation* is a method of expressing the degree of variation numerically.[1,6,7] If the biologic material follows the normal frequency distribution, 95 percent of all determinations on a sample will fall within two standard deviations of the mean.[1,6]

Standard deviation is expressed in the same unit in which values are measured. To have a common unit to compare the standard deviation of different tests it is useful to calculate the coefficient of variation.[6]** Two coefficients of variation are comparable to two standard deviations. Since the coefficient of variation is expressed in per cent, it is a useful tool in comparing the error of different procedures.

The conditions of our study were set up to conform as nearly as possible to our daily working conditions.

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* Standard deviation (S.D.) $= \sqrt{\frac{\Sigma d^2}{N-1}}$

N = Number of determinations done
d = Deviation of each value from the mean and may be a plus or minus value

** Coefficient of variation (C.V.) $= \frac{\text{Standard deviation}}{\text{Mean}} \times 100$ (in per cent)

† Read before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June 1958 and printed in the April 1959 issue of the Arkansas "Technicaller."

Methods

Blood for erythrocyte counts was collected with balanced ammonium potassium oxalate mixture as an anticoagulant. After the blood was well mixed by a mechanical shaker*, two red blood cell pipettes were filled. A pipette with a reputed accuracy of ±1 per cent was used. Random pipettes were calibrated in our laboratory and were found to contain volumes within ±1 per cent of the stated volumes. Using Hayem's solution as a diluent, a 1:200 dilution was made of the blood. After mixing at least three minutes on a Burton shaker and after discarding the first few drops from the pipette, two chambers of a hemocytometer using the Oren improved Neubauer ruling were filled from each pipette. Five groups of sixteen small squares or a total of eighty small squares were counted on each chamber. The four counts were averaged and the average count in millions, to the nearest tenth, reported. Even though in this study no counts were ruled out on the basis of cell distribution, it was found that all counts were acceptable according to the criteria of Ham.[6]

In the leukocyte studies blood was collected in balanced oxalate. White blood cell pipettes were used having an accuracy of ±1 per cent. A well mixed specimen of blood was diluted 1:20 with 0.1 normal hydrochloric acid. Burton shakers and hemocytometers with Oran improved Neubauer ruling were used.

Platelet counts were performed on two normal volunteers. Fresh finger punctures were performed for each count. All counts were collected as rapidly as possible. Dameshek's method of indirect platelet counting was used on one volunteer.[8] From each puncture two cover slip preparations were made from two drops of blood. Cover slips were

FIGURE II

Red Cell Count (Av. 4 Chambers, 2 Pipettes)	d (X-M)	d^2	Red Cell Count (Av. 4 Chambers, 2 Pipettes)	d (X-M)	d^2
455	36	1296	388	31	961
455	36	1296	426	7	49
418	1	1	437	15	225
414	5	25	400	19	361
414	5	25			
405	14	196	4606	..	5060
394	25	625			

$$N = 11$$

$$\frac{\Sigma X}{N} = \frac{4606}{11} = 419$$

$$1\,\text{S.D.} = \sqrt{\frac{\Sigma d^2}{N-1}} = \sqrt{\frac{5060}{10}} = 22.5$$

$$2\,\text{S.D.} = \pm 45 \text{ cells}$$

$$2\,\text{C.V.} = \frac{45}{419} \times 100 = \pm 11\%$$

* The mechanical shaker was a Kahn shaker adapted in our laboratory to mix hematology oxalate specimens.

rimmed with vaseline and platelets were counted per 1000 red blood cells on each of the preparations. The average of the two counts was reported. On the other volunteer direct platelet counts were done by the method of Brecher and Cronkite[3] using phase contrast microscopy. For this study red blood cell pipettes with ±1 per cent accuracy were used. A 1:200 dilution of blood with 1 per cent ammonium oxalate as the diluent was prepared. Two pipettes were collected per count. The twenty-five red blood cell squares or 1 square millimeter were counted per chamber. The total number of platelets seen on two chambers equaled the thousands of platelets per cu. mm. The final count was the result of the average of two pipettes.

Erythrocyte Counts

In one study eight persons took part, five technologists, one student technologist in the twelfth month of training and two laboratory aides. It was felt that the erythrocyte counts should not be entrusted to aides so they were included only as an experiment. Table I shows the standard deviation of the counts. Additional studies were performed on the blood of another normal volunteer and on the blood of a patient with a marked anemia. On each specimen we calculated the standard deviation of counts performed by the technologists alone, by the aides alone, and by both the technologists and aides. The mean counts by the aides alone showed no consistent difference when compared to the mean count of the technologists. All the laboratory aides had at least six months training in hematology just prior to the study.

A comparison of the coefficient of variation of normal erythrocyte counts and the decreased erythrocyte count revealed no great difference when counts were performed by both aides and technologists.

TABLE I—Standard Deviation of Erythrocyte Counts*

Blood Specimen	Persons Counting	Total Counts	Mean	2 S.D.	2 C.V.
A	M.T.**.....6	11	419	±45	±11%
	Aides.....2	4	394	±49	±12%
	M.T. + Aides...8	15	412	±50	±12%
B	M.T.....3	12	198	±26	±13%
	Aides.....4	12	195	±32	±16%
	M.T. + Aides...7	24	196	±27	±13.7%
C	M.T.....3	12	545	±60	±11%
	Aides.....4	12	585	±68	±12%
	M.T. + Aides...7	24	564	±79	±14%

* All counts on each individual performed from a single specimen of venous blood.
** M.T. include one student in the twelfth month of training.

Leukocyte Count

In the initial study the four large corner squares, each 1 sq. mm. in size, were counted on one chamber from each pipette. The total number of cells counted was used to calculate the deviation. Five technologists and two laboratory aides participated in this study.

Blood specimen D had a leukocyte count of approximately 5,000 per cu. mm. as shown in Table II. Two coefficients of variation equaled ±20 per cent. Specimen E had a leukocyte count of approximately 17,000 per cu. mm. and two coefficients of variation equaled ±17 per cent.

At a later date specimen F was studied. The leukocyte count was about 17,000 per cu. mm. The conditions of the test were the same except that the white cells counted on chambers 1 and 2 were averaged and taken to equal one count. Two coefficients of variation equaled ±18 per cent. Calculating the standard deviation on this same specimen using the count on chamber 1 only to equal one count two coefficients of variation equaled ±19 per cent.

TABLE II—Standard Deviation for Leukocyte Counts

Blood Specimen	Persons Counting	Chambers/Mean Pipette	2 S.D.	2 C.V.
D	M.T....5 Aides....2	1.....98	±20	±20%
E	M.T....5 Aides....2	1.....316	±50	±17%
F	M.T....5 Aides....3	2.....343	±64	±18%
F	M.T....5 Aides....3	1.....341	±66	±19%

Platelet Counts

Table III reveals the results of the studies of platelet counts. With four technologists performing twenty eight counts by the Dameshek method, two coefficients of variation equaled ±46%. The reproducibility of platelet counts by the method of Brecher and Cronkite was considerably better even though there was an additional technologist counting. The greatest reproducibility of counts was obtained with one technologist counting. In this instance the variation was only ±12% with a 95% confidence.

TABLE III—Comparison of Methods of Enumerating Platelets

Blood Sample	No. of M.T.	No. of Counts	Method	Mean	2 S.D.	2 C.V.
G	4	28	Dameshek	34/1000 RBC	±15	±46%
H	5	20	Phase	241	±62	±25%
I	1	7	Phase	286	±33	±12%

We found that allowing laboratory aides to count total erythrocyte and leukocyte counts did not significantly affect the reproducibility of counts done by the group of medical technologists in our laboratory that ordinarily performed this task. The calculation of standard deviation should be the project of each individual laboratory as is the calibration of colorimeters and other instruments. This type of statistical analysis can be invaluable in determining the range of normal values as well as the reproducibility of hematological and chemical procedures. The results may not live up to our expectations in so far as the error of the laboratory is involved but it may be seen where the greatest need for improvement exists. These results are not to be kept hidden in the laboratory files. They should be put into the hands of every physician using the laboratory facilities so that they may more realistically interpret laboratory results.

Summary

1. The importance of statistical analysis in evaluating laboratory data has been discussed. It was found that allowing the aides to assist with the erythrocyte and leukocyte counts did not significantly increase the error of results.
2. In our laboratory the direct method of counting platelets using the method of Brecher and Cronkite has been found to be more accurate than the indirect Dameshek method.

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ANNOUNCEMENT

There are vacancies for Medical Technologists in various Veterans Hospitals and Centers throughout the United States and Puerto Rico, the United States Civil Service Commission announces. The entrance salaries range from $4,980 to $7,030 a year.

Medical Technologists in these positions perform professional duties as technical assistants to pathologists in clinical and research laboratories of some 200 Veterans Administration hospitals and clinics. Applicants must have had appropriate education or a combination of education and experience. Students who are within 3 months of completing the required courses may apply.

Full information regarding the requirements and a list of places where applications are being accepted are contained in Announcement No. 194B. The announcement and application forms may be obtained at many post offices throughout the country, or from the U. S. Civil Service Commission, Washington 25, D. C. Applications will be accepted until further notice.

THE DETERMINATION OF CHOLESTEROL DIRECTLY UPON ACETONE-ETHANOL FILTRATES*†

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Zlatkis and co-workers[12] described a method for the determination of cholesterol by treating serum directly with ferric chloride, glacial acetic acid and sulfuric acid. However, it was quickly recognized that tryptohan[8], steroids[1], bromides[5], Vitamin A[3] and long-chain unsaturated fatty acids[4] as well as cholesterol produce color in this reaction. Therefore, attempts have been made to eliminate some of these substances from the reaction mixture. Henly[2] modified the original technique so that proteins are removed by precipitation with the ferric chloride dissolved in glacial acetic acid prior to treatment with sulfuric acid. Recently, Zak[11] has proposed the extraction of cholesterol from serum with acetone-ethanol and solvent evaporation before color production. The drying procedure is lengthy and must be carefully controlled. Therefore, a technique has been devised which treats aliquots of the acetone-ethanol filtrate directly with the color reagents. This modification shortens the existing procedures considerably and provides less opportunity for loss of the precipitate.

MATERIALS

Ferric chloride-glacial acetic acid reagent. A 0.05% solution is prepared by diluting 500 mg. of $FeCl_3 \cdot 6H_2O$ to 1 liter with reagent grade glacial acetic acid.

Concentrated sulfuric acid. An analytical reagent grade.

Digitonin solution. A 0.5% solution is prepared by dissolving 1.0 gm. of digitonin in 100 ml. of absolute ethanol at 60°C. After the addition of 2.0 ml. of 10% acetic acid the mixture is diluted to 200 ml. with distilled water.

Acetone-ethanol. A 1:1 mixture is prepared.

Acetone-ether. A 1:1 mixture is prepared.

Cholesterol standard. A 60 mg% standard is prepared by diluting 120 mg. of purified cholesterol to 200 ml. with the acetone-ethanol mixture. The solution is kept tightly stoppered and renewed frequently.

METHODS

Determination of serum total cholesterol. Quantitatively transfer 0.3 ml. of serum into 2.7 ml. of acetone-ethanol. Stopper, mix well and centrifuge. Place 0.3 ml. of clear filtrate in a colorimeter tube and add 5.0 ml. of ferric chloride-glacial acetic acid reagent. Blow in with force 3.0 ml. of concentrated sulfuric acid. If complete mixing is not obtained a small glass stirring rod may be used to obtain uniformity. Allow 10 minutes to elapse before reading so that the reaction mixtures are completely free from bubbles. The optical densities can be measured between 540 and 560 mu. against a reagent blank containing 0.3 ml. of acetone-ethanol instead of serum filtrate.

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Determination of serum free cholesterol. Transfer 1.0 ml. of serum filtrate to a 15 ml. conical centrifuge tube. Add 1.0 ml. of digitonin solution, mix well and allow to precipitate for at least 1 hour. Centrifuge, decant the supernatant and suspend the precipitate in acetone-ether. Centrifuge and carefully discard the supernatant. Dilute the cholesterol digitonide with 5.0 ml. of the ferric chloride reagent. Dissolving is often facilitated by warming at 37°C. for a short time. When the precipitate is completely in solution, cool to room temperature and blow in 3.0 ml. of concentrated sulfuric acid. Transfer the reaction mixture to a colorimeter tube and read as described above.

Standardization for total cholesterol and cholesterol digitonides. Transfer 0.1, 0.2, and 0.3 ml. of the stock cholesterol solution to colorimeter tubes. Place similar portions in conical centrifuge tubes for precipitation as digitonides. For the total cholesterol measurements these dilutions are equivalent to 200, 400 and 600 mg% cholesterol respectively. When 1.0 ml. of acetone-ethanol filtrate is treated with digitonin these standards are equivalent to 60, 120 and 180 mg% cholesterol digitonide. Dilute the total cholesterol standards to 0.3 ml. with acetone-ethanol before adding 5.0 ml. of the color reagent. Isolate the cholesterol digitonides and dilute with a similar amount of color reagent. Thoroughly mix all solutions with 3.0 ml. of sulfuric acid and read after 10 minutes against a reagent blank between 540 and 560 mu.

RESULTS AND DISCUSSION

Filtrates obtained by adding serum directly to the ferric chloride reagent according to Henly's method[2] often produced colors which differed markedly from that of the cholesterol standards. When sulfuric acid was layered for several seconds with the ferric chloride-acetic acid filtrate a chromogen developed at the interface. This appeared to be due to the formation of a brown substance in the filtrate. Robinson and Pugh[6] have suggested that even minor charring at the acid-color reagent interface may result in errors which are difficult to detect. A comparison of the absorption curves produced by pure cholesterol and a serum filtrate obtained with the ferric chloride reagent was made on a Coleman Universal spectrophotometer. (Figure I). The cholesterol standard shows a well-defined peak between 540 and 560 mu. The serum filtrate absorbs most strongly in the spectral region near 500 mu. with no peak exhibited between 540 and 560 mu. Therefore, it may be impossible to quantitate cholesterol levels in the absence of the required absorption maximum.

Acetone-ethanol filtrates of the same serum specimens were prepared in an attempt to eliminate substances producing the interfering chromogens. Other workers[7,9,10] using acetone-ethanol for cholesterol isolation have dried the extracts for long periods of time. To eliminate the time consuming evaporation procedure 0.3 ml. of the 1 to 10 acetone-ethanol serum filtrates were treated directly with the color reagent and sulfuric acid. A similar amount of acetone-ethanol was added to the cholesterol standards and the absorption curves obtained were compared. (Figure II). The absorption curves were similar between 400 and 700 mu. as measured on the Coleman Universal spectrophotometer. No

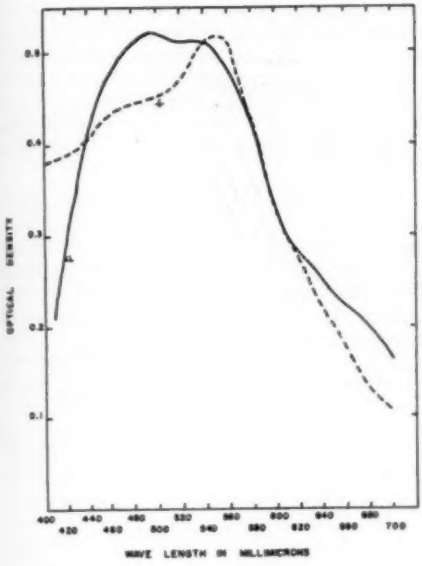

Fig. I

A comparison of absorption curves obtained using ferric chloride-acetic acid filtrates with (a) cholesterol from serum and (b) purified cholesterol.

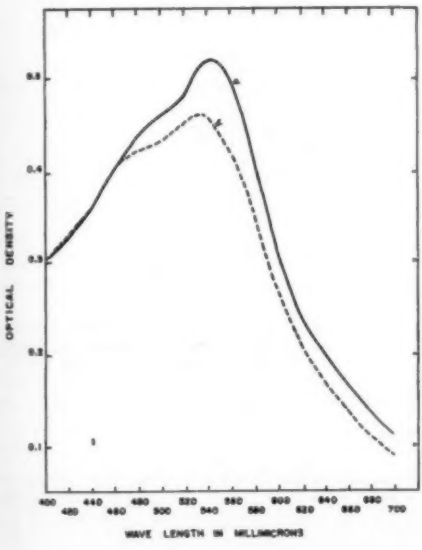

Fig. II

A comparison of absorption curves obtained with (a) purified cholesterol and (b) serum cholesterol in the presence of 0.3 ml. of acetone-ethanol.

evidence of discoloration due to charring was observed using the acetone-ethanol extracts. This suggests that the interfering substances are not extracted from serum by use of this solvent mixture. The efficiency of protein precipitation may be an important factor in the charring. The use of ferric chloride-glacial acetic acid as a protein precipitant often required vigorous mixing, warming or storage overnight to obtain clear supernatants. A more rapid and complete protein separation occurred by use of the acetone-ethanol.

A standard dilution curve was prepared to determine whether cholesterol could be quantitated when 0.3 ml. of acetone-ethanol was added to the reaction mixture. (Figure III) demonstrates that the chromogen formation was linear between cholesterol concentrations of 40 to 200 ug. regardless of the presence of the lipid solvent. Although acetone-ethanol lessens color development some 20% it is still possible to accurately quantitate cholesterol levels up to 650 mg%.

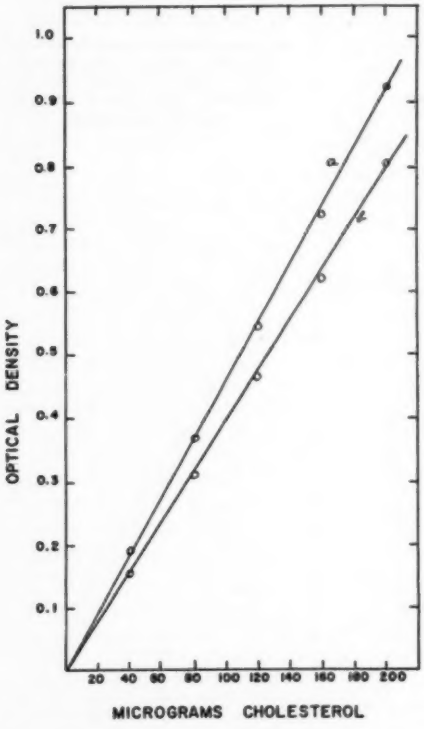

Fig. III
A standard curve utilizing purified cholesterol (a) without acetone-ethanol and (b) with 0.3 ml. of the solvent.

Recoveries of total and free cholesterol employing the proposed method were examined by adding various amounts of purified cholesterol to a pooled serum. (Table I). The amounts of total and free

TABLE I

Total and Free Cholesterol Recoveries

Serum Sample	Free Cholesterol Added (mg. %)	Total Cholesterol Present (mg. %)	Total Free Cholesterol Present (mg. %)	Total Cholesterol Recovered (mg. %)	Total Free Cholesterol Recovered (mg. %)
A	0	235	50	...	...
B	40	275	90	271[1]	92
C	80	315	130	316	134
D	120	355	170	356	167
E	160	395	210	400	206
F	200	435	250	432	250
G	280	515	330	522	332
H	320	555	370	557	378

[1] Each figure given for a cholesterol recovery represents an average of two determinations.

cholesterol found by averaging two single determinations were within 7 mg% of the theoretical values. The precision obtainable with the new technique was investigated utilizing 24 randomly selected sera from hospital patients. (Table II) Many of these specimens were icteric or lipemic and several were hemolyzed. Two total and three free cholesterol determinations were carried out on each specimen. The single total cholesterol measurements did not vary by more than 11 mg% from each other. The average difference amounted to only 4 mg% cholesterol. Acceptable precision was also obtained on the three free cholesterol determinations.

TABLE II

Replicate Total and Free Cholesterol Determinations

Sample	Total Cholesterol (mg. %)		Free Cholesterol (mg. %)			Sample	Total Cholesterol (mg. %)		Free Cholesterol (mg. %)		
A	207	205	55	59	59	M	164	164	78	78	76
B	185	180	58	60	60	N	371	371	136	136	138
C	268	259	76	78	78	O	235	230	80	81	84
D	202	191	60	60	58	P	265	267	95	95	94
E	251	253	86	86	84	Q	350	350	126	127	128
F	216	208	42	42	44	R	367	362	90	92	94
G	306	306	76	77	78	S	317	322	112	115	115
H	191	196	44	46	46	T	223	219	86	86	84
I	246	254	71	68	73	U	177	170	54	55	60
J	257	262	71	71	72	V	134	134	51	51	51
K	207	202	50	47	48	W	278	275	112	115	112
L	202	207	50	52	55	X	101	103	52	52	54

To establish normal values for total and free cholesterol using the proposed technique serum samples were obtained from 100 blood donors. The average total cholesterol was 186 mg% with a range of 113 to 362 mg%. It is generally accepted that the normal range is from 150 to 240 mg% with an occasional figure above and below these values. The free cholesterol varied from 19% to 40% of the total with an average of 27%. By other methods it has been established that free cholesterol normally forms about 30% of the total with a range of 20 to 40%.

SUMMARY

Cholesterol extracted directly from serum with ferric chloride-glacial acetic acid often produces brown chromogens with sulfuric acid. The isolation of cholesterol with acetone-ethanol prior to color formation makes charring much less likely. Direct treatment of this lipid extract with the ferric chloride reagent and sulfuric acid greatly reduces the test time. Results obtained by use of this technique for total and free cholesterol on 100 blood donors compare favorably with acceptable normal values. The precision and recoveries obtainable make the proposed procedure rapid and reliable for clinical and research analyses.

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CONSTRUCTION AND USE OF NOMOGRAMS FOR CLINICAL CHEMISTRY*

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A nomogram,[2,5] in the broadest sense, is any device which states a mathematical law in graphic form. The possibilities for the use of nomograms to simplify computations are almost unlimited, and extensive use has been made of them in some fields, notably chemical and mechanical engineering. But in the field of medicine and clinical laboratory work the use of nomograms has been sparse indeed.[1] This is surprising in view of the many calculations which must be made daily in clinical chemistry laboratories.

Of course, some problems can be solved quickly with a slide-rule. However, many technologists are unable or unwilling to use slide-rules or are not provided with them. For these technologists some other simple and inexpensive aid to calculation would be a great help. Furthermore, calculations requiring an addition or substraction step between multiplications or divisions cannot be done directly on a slide-rule. Precious time is gobbled up with lengthy calculations from complicated formulae and errors are likely to creep into such multiple-step operations.

One of the greatest advantages to the use of nomograms is that they invariably yield the same result for a given set of readings. It is a fact that in hand calculations there will be simple arithmetical errors occurring in a certain small percentage of cases. The use of well constructed nomograms can reduce such errors to an absolute minimum, since one need only to transfer the readings from the photoelectric colorimeter or spectrophotometer to the nomogram and read off the result. The value of nomograms as time savers is even more obvious. In our laboratory, the time for calculations on tests for which we have made nomograms has been reduced to one-fourth of what it was formerly.

It is the purpose of this paper to present and illustrate methods for the construction of simple nomograms suitable for different types of calculations encountered in clinical chemistry.

Materials:

1. Graph paper
2. Ruler—preferably graduated in tenths of inches
3. Soft wallboard or corkboard
4. Round headed pins
5. Thread—red or black

Methods:

In all of the nomograms described below a pin and thread are used in place of a straightedge. This simple device is much more convenient to use than the transparent straightedges usually recommended, since both ends are firmly fixed at once, and the hands are free to record results without disturbing the setup. The nomograms are mounted on a soft wallboard or corkboard which will admit the pins easily, yet hold them firmly.

I. Nomograms for colorimetric determinations which follow Beer's Law:

A. Tests run without standards, based on an original calibration checked only occasionally. Calculations involve only multiplication of readings by a factor.

*Read before the 27th Annual Convention of ASMT, Phoenix, Arizona, June, 1959.

B. Tests run with standards in each batch from which values are calculated by use of the relation:

$$\frac{\text{O. D. of Unknown}}{\text{O. D. of Standard}} \times \text{conc. of std.} = \text{conc. of Unknown}$$

Figure 1 shows how a single nomogram can be made for several different determinations of both types. One pin with thread attached is pushed in at the zero point and left there. To use the chart for a Type A calculation, simply pull the thread taut and rotate it until it crosses the spot marked for the appropriate test. Then push the pin at the other end into the mounting. It is now read like an ordinary graph except that the decimal point must be inserted in the proper place in the answer. The dotted line in Fig. 1 shows such a set-up for a microbilirubin (M. bil.) in which optical density readings are divided by .0225. To locate this point when making the chart, merely pick a convenient large value, say 20mg., and place a red spot on the 20 mg. line (200 on the graph) opposite its corresponding optical density (.450).

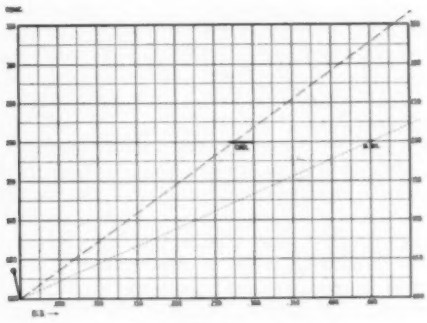


Fig. 1

For Type B computations in which there is a range of acceptable standard readings, a line is drawn in red representing this range on the horizontal line which equals the concentration of the standard. The solid line on Fig. 1 labeled "Chol." represents a standard of 200 mg. with acceptable readings from .270 to .300. To use the chart for cholesterols it is necessary only to place the thread so as to cross the "chol" line at the optical density value for the standard. In Fig. 1 the dashed line shows a set-up in which the standard read .275.

II. Nomograms for miscellaneous calculations:

Details of the construction of two different types of nomograms will be discussed here. A mathematical approach and an empircal approach are given. It should not be inferred that the methods described are the only ones available, but they are relatively simple and the principles involved may be easily applied to many other problems.

Type I: For equations of the general form A + B = C.
The example illustrated in Fig. 2 is a nomogram of Type 1. It is designed for the BSP method of Gaebler[3] which involves the following equation:

$$\frac{\text{O. D. at } 565_m - (1.3 \times \text{O. D. at } 620_m)}{K} = \% \text{ dye retained}$$

K is a contant which depends on the instrument used and is determined when the method is set up In our laboratory it is 0.00628 and the nomogram in Fig. 2 is based on that figure. If the equation above is arranged thusly:

$$\underset{(A)}{\frac{\text{O. D. at } 565_m}{.00628}} + \underset{(B)}{\frac{-1.3 \times \text{O. D. at } 620_m}{.00628}} = \underset{(C)}{\% \text{ dye retained}}$$

it is clear that it falls into Type 1 category. Each term in the equation is represented by a scale on the nomogram. It is necessary to select first the dimensions of the nomogram (10" x 7.62") and the range of possible or likely readings for O. D. at 565_m and O.D. at 620_m (0 to 1.000 and 0 to .250 respectively). It is a simple matter to construct two 10 in. scales for these ranges. The problem is to determine the proper distance relations between these scales and the read-out scale. Distance d is defined by the relation $d = \frac{b}{b + c} W$ where W is the width (7.62") and b and c are factors for scales B and C respectively. b is found by dividing the height (10") by the difference between the highest and the lowest values for the term $B \frac{(1.3 \times .250)}{(.00628)}$. c is found similarly, 10" divided by 107.3 (value of C at highest O. D.'s minus value at lowest). On calculating, d is found to be 5.14". Naturally, the same procedure can be used to make a nomogram for different K values merely by substitution of the other K value for the .00628 used here.

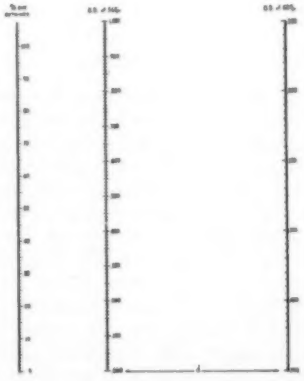


Fig. 2

Type 2: For equations of the general form $A \cdot B = C$.
Fig. 3 shows a simple nomogram of this type for the cephalin-cholesterol flocculation test of Kibrick,[4] which involves the following equation:

$$\frac{\text{O. D. of control} - \text{O. D. of patient} \times 100}{\text{O. D. of control}} = \% \text{ ceph. floc.}$$

If rewritten thusly: $\left[\frac{1}{1 - (P \cdot \frac{1}{C})}\right] \times 100 = \%$ ceph. floc. it is a Type 2 equation. The general form for Type 2 nomograms is an N shape with the two vertical scales linear and the slanting one non-linear and meeting the other two scales at their zero points. Since there is but one control reading for each run, the pin is pushed in the proper place on the "control" scale and left there. The thread is then moved so as to cross the "patient" scale at the appropriate optical density and the result read on the "% C.F." scale which is graduated downward.

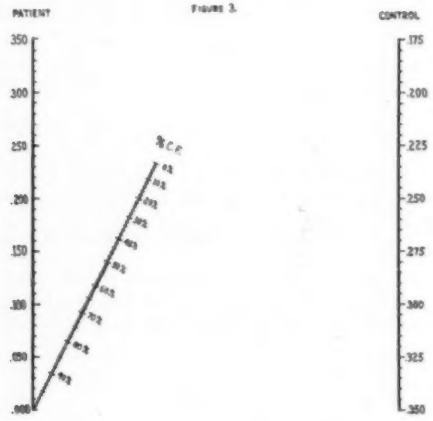
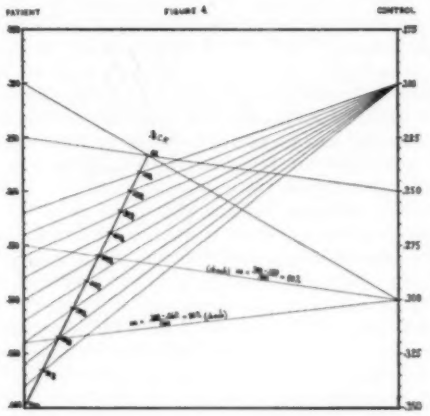


FIGURE 3.

To make this chart, a scale of convenient size reading downward from .175 to .350 is set up vertically toward the right hand side of the paper to represent the control scale (Fig. 4). The "patient" scale, of the same height but reading upward from zero to .350, is placed to the left of the control scale at a distance roughly equal to its height. (This distance is not crucial and need not even be measured.) Now connect any two values on one scale with the corresponding values on the other scale. The point of intersection will be the 0% end of the diagonal scale. Connect this point with the zero end of the "patient" scale. It is necessary to locate the major intervening points in the same manner since the diagonal scale will not be uniform. Pick a number on the control scale which will make the computations simple, say .200. Connect .200 on the control scale with .180 on the "patient" scale, mark where it crosses the %C. F. scale and label 10%. Now move to .160 on the "patient" scale to locate 20%

and so on to 100%. The remaining graduations may be added with sufficient accuracy by merely dividing each segment into ten equal parts. Naturally, any new nomogram should be checked with many random sets of readings to be sure it is correctly laid out.



Summary:

Graphic methods for computations save time and reduce errors. Much more use can be made of them in clinical laboratories. Methods are described here for making nomograms for tests which follow Beer's Law, whether or not standards are used in each run. Also, two general types of nomograms for special calculations are described and illustrated. Details of construction are shown, using a mathematical approach for one nomogram and an empirical approach to the other.

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MEDICAL TECHNOLOGIST—PROFESSIONAL*

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More than 12 years ago, in an address delivered before the Engineering Institute of Canada, William E. Wickenden, an electrical engineer with honorary degrees from nine institutions, stated that "every calling has its mile of compulsion, its daily round of tasks and duties, its standards of honest craftsmanship, its code of man-to-man relations, which one must cover if he is to survive. Beyond that lies the mile of voluntary effort, where men strive for excellence, give unrequited service for the common good, and seek to invest their work with a wide and enduring significance. It is only in this second mile that a calling may attain to the dignity and the distinction of a profession."

Strange—or is it so strange—that each of us takes a certain pride in calling himself "professional"? Just what is the attraction of the term? I am inclined to believe it is basically that each of us finds greater satisfaction in something that requires just a bit more effort, that entails slightly more of a sense of responsibility, and a little more skill than ordinary to accomplish. With personal satisfaction comes that feeling of sharing with the group—the profession—inherently common interests, common standards, ethics, and qualifications, with the implication that these are all on a high level.

Somehow, rather than coming out as a "brand new" entity, the medical technologist has undergone a rather slow and painful growth from the "lab girl," to the "laboratory technician," to the "medical technician," and, finally, into the status of the professional medical technologist. Much of this far-from-fast development is due to the very nature of the members of the "calling"—rather introspective, conscientious, fairly retiring, willing, even anxious to work alone and in the background, yet more than a little resentful that others often don't seem to recognize their value, their importance in the scheme of things as related to the medical world.

Even now, many medical technologists, although theirs is a healthy, growing and progressive association, fail to recognize their own professional responsibilities—that their participation is necessary if medical technology is to gain "professional recognition." The American Society of Medical Technologists has over 8,000 members, but again as many medical technologists eligible for membership fail to take advantage of association with others who have the same interests, and who "do something about it." In order to be an active member of the A.S.M.T., one must either be certified by the Registry of Medical Technologists of the American Society of Clinical Pathologists, or must have at least a Master's degree in one of the fields of medical technology; serology, microbiology, hematology, biochemistry, histology, et cetera. Unlike those of most of the other para-medical groups, the certifying body for medical technologists is a separate unit from their professional association. The Registry of Medical Technologists is an autonomous "committee" made

* Reprinted upon request with permission from HOSPITAL MANAGEMENT, March, 1959, page 36.

up of persons representing both the American Society of Medical Technologists and the American Society of Clinical Pathologists.

The relationship of medical technology to the branch of medicine with which it is most closely associated: namely, pathology, is a most fortunate one from the standpoint of both groups, especially in the matter of well integrated recruitment and training programs. The only danger of this close relationship lies in the possible failure of the medical technologist to recognize his own responsibilities and so become too dependent upon the pathologist to solve all the problems of the technologist, to depend upon the pathologist to serve as the intermediary for all his hospital relationships, while the technologist harbors an inward resentment because he is "not respected." This type of situation is, fortunately, slowly becoming resolved. As the medical technologist himself is maturing, more "career" technologists are in the picture. Many women who have reared their families are taking "refresher" courses and are returning to work. With additional college requirements for entering American Medical Association Approved Schools of Medical Technology, a more responsible and mature medical technologist is emerging from those schools.

With the increasing complexity of laboratory procedures, the medical technologist, himself, is recognizing the need for additional college or university background. There is a greater demand for graduate courses in medical technology, per se, as well as for those in the related areas. Each year there are more refresher courses and seminars programmed under the sponsorship of medical schools, universities, and state societies of medical technologists.

Altogether, that unity of medical technologists who comprise the membership of the American Society of Medical Technologists can well consider themselves professional colleagues. May the tribe increase, and grow, and mature. Professionalism is a matter, primarily, of attitude.

A SIMPLIFIED METHOD FOR THE DETERMINATION OF SERUM BILIRUBIN IN THE ROUTINE CLINICAL LABORATORY*

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The need for a micro method for measuring bilirubin concentration in serum or plasma has been recognized by a number of workers and a number of methods have been described (1, 2, 3, 6). The most obvious use of such a method is in the case of suspected hemolytic disease of the newborn. Generally, the micro methods have been modifications of diazotinazation or other macro methods, involving the customary series of chemical manipulations.

Recently, White et al (7) described a simple method for determining bilirubin. Unfortunately, a Beckman Du Spectrophotometer was recommended. Such an instrument is not ordinarily used in some routine clinical laboratories; simpler and less expensive instruments are preferred. Although it was indicated that filter photometers or spectrophotometers ordinarily used in the clinical laboratory were unsuitable (7), no trial of the method had been attempted using the Bausch and Lomb Spectronic 20 (5), the band width of which lies midway between the Beckman and other instruments referred to. Furthermore, these authors had stressed the use of capillary tubes, whose standardization, preparation and use usually involved measuring, weighing, calculating, rinsing, testing for free alkali and/or drying.

It was believed at this laboratory that the usefulness of this simple micro method would be greatly extended if clinically significant results could be obtained using the Spectronic 20 and lambda pipets. Accordingly, a preliminary comparative study was undertaken on sera.

MATERIALS AND METHODS

Micro Method

Sera were sampled with 50 lambda (0.05 ml) pipets. Optical Density was read in the Bausch and Lomb Spectronic 20. This instrument was equipped with a micro cell carrier (A. H. Thomas Co., Philadelphia, Pa., accessory for the B & L Spectronic 20, No. 9085-D1) and micro cuvettes (A. H. Thomas Co., accessories for the B & L Spectronic 20, cuvette No. 9085 N-2 with cover No. 9085 N-5) with a 10 mm light path.

Procedure:

1. Place 1.0 ml of buffer into a micro cuvette.
2. Add 50 lambda serum (or standard), rinsing the pipet several times with the buffer in the cuvette. Mix contents thoroughly, by placing the cover on the cuvette and inverting several times.
3. Read Optical Density at both 455 mu and 575 mu. The instrument must be zeroed separately at each wave length with water and buffer.

*Received for publication April 1959.

Calculation:

$$\frac{\text{O.D.}_{455} - \text{O.D.}_{575}}{0.05 \text{ (Volume of sample in ml)}} \times 1.4 \text{ (constant of White et al)} = \text{mgm \%}$$

Buffer: Dissolve —7.65 grams $Na_2HPO_4 \cdot 7H_2O$ and 1.74 grams KH_2PO_4 in a liter of water. The pH should be about 7.4.

Standard:

(1) Accurately weigh 16.0 mgm of pure bilirubin. To this add 4.0 ml of alkaline solvent solution (pH 11.3), prepared by diluting 1.0 ml of 5N NaOH to 100 ml with M/15 Sorenson buffer (pH 7.4). The alkaline solvent should be freshly prepared before use. After 4 to 5 minutes, the mixture is gently stirred with a glass rod until the bilirubin is dissolved, and 10 ml of 25% normal human albumin is added. Transfer the solution to a 100 ml volumetric flask with washing and make up to volume with Sorenson buffer. More dilute standards may be prepared from this one (16.0 mgm %) by using Sorenson buffer as diluent. The final solution should have a pH of 7.3-7.5.

(2) The standard solutions are kept in a deep freeze in small (2 ml) portions until used. A bilirubin standard of appropriate concentration should be run along with the unknown sera, to act as a known control.*

(3) A frozen serum of known bilirubin concentration may also be used as a control. It is less costly, but probably not as accurate as the synthetic control.

Collection of Sample:

Sera used in this study were usually obtained from venous or cord blood. In routine practice, however, sufficient serum may be obtained by filling 3 to 5 capillary tubes from a finger or heel puncture. These tubes are available commercially; No. 51661, micro blood collecting tubes, plain (Scientific Products Division, American Hospital Supply Corp., Evanston, Illinois) are used at this laboratory. One end of each tube is sealed in a small flame and centrifuged. If a micro centrifuge capable of carrying the large size capillary tube (150 mm long) is not available, the sealed tubes may be placed in a 100x15 mm test tube and centrifuged at 2000 rpm for 10 minutes. Following centrifugation the tubes are broken off just above the junction of the serum and cell columns. The serum is collected in a 75x10 mm test tube, from which it can be removed easily with a lambda pipet.

Macro Method

The same sera were also assayed for bilirubin using the conventional macro diazotization method of Malloy and Evelyn (4).

RESULTS

Data comparing the two methods are presented in Table 1. The results obtained by the modified micro method compare very favorably with the values obtained using the standard macro procedure. Some possible reasons for an occasional difference are discussed below.

DISCUSSION

Even though the two methods used in this study depend upon different principles, results are sufficiently comparable to have clinical meaning. This is particularly true in the higher range of values. One might note at this point that most bilirubin methods tend to be less accurate when lower concentrations (below 1 or 2 mgm %) are encountered.

As pointed out by White et al (7), lipochrome substances might be

* The frozen standard solutions are stable for about six weeks or longer. Deterioration is shown by the color—of the frozen solutions changing from yellow to green.

TABLE I

Serum bilirubin assay by the macro and modified micro methods

ADULTS		NEWBORNS		ADULTS		NEWBORNS	
Macro mgm %	Micro mgm %	Macro mgm %	Micro mgm %	Macro mgm %	Micro mgm %	Macro mgm %	Micro mgm %
0.35	0.42	0.25	0.15	2.35	2.24	3.50	3.30
0.40	0.42	0.35	0.30	3.50	3.50	3.70	3.50
0.50	0.56	1.20	1.25	3.60	2.80	7.20	8.30
0.55	0.70	1.60	1.60	3.80	3.92	8.00	8.40
0.55	0.84	1.90	1.96	5.15	5.04	8.35	9.53
0.65	1.12	2.00	2.23	5.80	5.60	13.6	14.2
1.60	1.54	2.05	3.00	9 50	8.96	15.3	16.8
1.75	1.88	2.20	2.20	11.9	13.1	20.2	19.6
1.75	3.64	2.30	2.50	22.5	23.0	22.0	22.7
2.05	1.96	3.00	2.90	33.3	31.3	....	....
2.15	2.24	3.35	3.10				

present in adult blood, with a resulting increase in absorbance at 455 mu, thus giving a false higher value with the micro method. This may account for a few situations in our comparative data where the values obtained by the micro method are significantly higher than the macro results, and might be a limiting factor in the general use of the micro method. However, no such error is likely to occur in the case of the newborn, where this micro method would be of greatest value. White and his coworkers also stated that, in their experience, the presence of more than minimal amounts of hemoglobin gave falsely low bilirubin results by the diazo reaction. Their micro method, however, is supposed to correct for the presence of the more commonly encountered heme pigments.

SUMMARY

An adaption of the micro method of White, Haidar and Reinhold for the determination of bilirubin in infants has been described using the Bausch and Lomb Spectronic 20 and lambda pipets. Thus modified, this method can be sufficiently simple, inexpensive and accurate to be employed in any routine clinical laboratory possessing such equipment. Data are presented to support this conclusion.

ACKNOWLEDGEMENT

The authors wish to thank Dr. J. G. Reinhold, of the William Pepper Laboratory of the University of Pennsylvania, for his cooperation in providing a sample of a bilirubin standard used at that institution, as well as details for the preparation of such standards.

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DIAGNOSTIC BACTERIOLOGY OF SPINAL FLUID*

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Meningitis is an inflammation of the meninges or coverings of the brain. There are a number of types of meningeal reactions but those arising from frank infections will be discussed.

Meningitis may be roughly divided into two main groups, the non-suppurative and the suppurative. The non-suppurative groups of meningeal reactions usually result in cell counts below 2000 per cmm. with a predominance of lymphocytes. Of these tuberculous meningitis, mycotic infections of the brain, leptospirosis involving the meninges and parasite infections concern us. The suppurative group is meningitis caused by pus-producing organisms and whose meningeal reactions result generally in high cell counts, predominately polymorphonuclear cells. The epidemic type of meningitis is caused by *N. intracellularis. H. Influenzae* and *D. Pneumoniae* are the other two organisms most commonly encountered, but Streptococci, Staphylococci, enteric organisms, Bacterioides group, *Pseudomonas aeruginosa.* Proteus groups, Listeria and other organisms may be the causative agent.

Due to the acute nature of this disease, speed in diagnosis is very important. Cerebrospinal fluid is the specimen used for bacteriological study. It is obtained by lumbar puncture and must be collected in sterile containers.

At our hospital, all cultures of spinal fluid are made at the bedside. Spinal fluid is dripped directly from the puncture needle into the media. Four types of media are used; 0.2% agar glucose tryptose semi-solid, dispensed in 20 cc. amounts in screw-capped tubes; a blood and chocolate slant in screw-capped tubes, and thiol medium. The thiol medium is especially prepared to neutralize the antibiotics which might be in the spinal fluid. The heavy semi-solid used, gives us a tube of medium in which we may grow anaerobic organisms at the bottom and have all degrees of oxygen tension available so that CO_2 jars are not required. Meningococci for instance, grow luxuriantly just below the surface of the medium.

We have set up a criterion for when and how the spinal fluids should be cultured at the bedside.

1. If the patient has a diagnosed virus infection—as frankly diagnosed poliomyelitis—no cultures are made.
2. An undiagnosed case but suspected of viral origin with clear spinal fluid is cultured in the semi-solid medium only.
3. Spinal fluids from tuberculous meningitis are cultured in semi-solid agar. This is done to rule out the possibility of a very early or superimposed purulent meningitis. A culture is always made in semi-solid media following any spinal tap done on these patients. This is useful as an early check for secondary infections which might occur during repeated spinal taps.
4. All cloudy spinal fluids, regardless of clinical diagnosis, and all spinal fluids from cases suspected of being suppurative meningitis are cultured directly into the four types of media.

The cultures are incubated at 37° C immediately. They are examined daily until growth appears grossly. Smears are prepared, gram-stained

* Reprinted from the Michigan Bulletin for Medical Technologists, May 1955.

and examined microscopically. Transfers are made from semi-solid agar and thiol medium to blood agar plates or other types of media as necessary. The organisms are identified using standard bacteriological methods. If no growth apppears by the seventh day, smears are prepared from the culture, and if no organisms are seen, a report of no growth is recorded. Occasionally cultures must be kept longer than seven days if the history of the patient seems to indicate the necessity.

All cloudy spinal fluids collected in autoclaved screw-capped tubes are brought to the laboratory as quickly as possible for direct smear examination. The fluid is centrifuged at 2500 RPM's for 10 minutes. Smears of the sediment are gram stained. Care must be taken in decolorizing the gram stain, since the cells in the spinal fluid tend to hold the crystal violet and make it difficult to read. Morphology and gram's reaction are reported from these smears.

If organisms are seen on direct smear, sensitivity tests using Difco disks are immediately set up. These are read as soon as sufficient growth appears to give an accurate answer. Sometimes this may be accomplished in from six to eight hours. If no organisms are seen on direct smear, the sensitivity tests are set up as fast as possible from the cultures. Cultures received in the morning are usually ready for sensitivity tests by late afternoon.

Gram negative biscuit-shaped diplococci are never reported *N. intracellularis,* since about 1.5% of the gram negative diplococci of meningitis is *N. gonorrheae* and another 1.5% may be one of the other Neisseria groups. *N. sicca* may be encountered infrequently. As soon as enough growth of gram negative diplococci is obtained, and this need be only one or two colonies, a slide agglutination test is done using antimeningococci sera. Groups A, B, and C. Then the organisms are reported as *N. intracellularis* Group— according to the agglutination. Those, which do not agglutinate, must be identified by their carbohydrate fermentations. Glucose, maltose and saccharose prepared in Phenol Red broth to which is added 0.2% agar is used.

On all atypical gram negative diplococci, a Quellung test using antipneumococci sera is performed. In spinal fluids from patients who have had antibiotic treatments, the pneumococci often lose their gram staining properties and become gram negative. This loss may remain for one or even two transplants but will eventually return to gram positive. Since Quellung reaction is specific, these organisms are reported directly by name and type. This is important, for the treatment of pneumococcal meningitis necessitates much larger doses of antibiotics than the Neisseria groups. All gram positive cocci and diplococci seen on direct smear are set up for Quellung. In our experience, the morphology of pneumococci in spinal fluids is variable, so not too much reliance can be placed on it. When Quellung occurs the organism is reported immediately by name and type. If the organisms do not quell and on culture show alpha hemolysis on blood, a bile solubility test is done. Since we only have 45 antisera, it may be a higher type of pneumococcus. All other gram positive cocci are identified by the usual bacteriological procedures.

All gram negative rods seen on direct smear from spinal fluids, regardless of morphology and size, are set up for Quellung reaction using antiinfluenzae B serum. If Quellung occurs, they are immediately re-

ported as *H. influenzae B*. It has been found that *H. influenzae B* assumes many bizarre forms in spinal fluid. Immediate identification is important since the treatment for meningitis caused by the gram negative rods differs from that for the gram negative diplococci and the gram positive groups. If no Quellung occurs and the rods are reasonably suggestive, Quellung tests are done against Freidlanders antisera A, B, and C. Again a positive Quellung reaction identifies the organism. All other gram negative rods must be identified following the usual bacteriological methods.

When a patient has a meningitis with a gram negative rod as the causative agent, before treatment is discontinued, even if the physical condition of the patient is good, the spinal fluid must be free of organisms. Direct smears are prepared and examined very carefully, for the organisms are often few in number. If organisms are found, even though they may not grow, treatment is continued. It has been observed that if organisms are found and treatment has been stopped, the patient often has a relapse. If no organisms are seen the patient is taken off treatment for a week. Another tap is done at this time and if no organisms are seen, the patient is released.

All organisms grown from spinal fluid cultures are identified and reported. The doctor must decide whether the organisms may be a contaminant or not. When doubt occurs, repeat cultures are made.

Since antibiotic and chemotherapy has been instigated for tuberculous meningitis and due to a changing concept of this disease, a presumptive diagnosis made by finding the acid fast rods as soon as possible is paramount. Atypical cases occur and early treatment is imperative. Since the reporting of acid fast rods in spinal fluid may mean months of hospitalization for the patient, frequent checks on slides and stains must be done. The slides must be clean and the stains uncontaminated. Slides are stained separately.

Two tubes of spinal fluid in sterile tubes are sent to the bacteriology laboratory from patients suspected of having tuberculous meningitis. One is for direct smear examination and the other to culture for the tubercle bacillus. If animal inoculation is requested a third tube must be sent. As much spinal fluid as possible is desired—at least 5cc., preferably more.

For direct smear examination, equal parts of 95% alcohol is added to the spinal fluid to precipitate the protein. It is allowed to stand overnight if possible. Since the spinal fluid is colloidal in nature, without precipitation, the organisms do not centrifuge down well. The precipitated spinal fluid is centrifuged at 2500 RPM for ½ hour and all the sediment used for smears. The smears are stained by the Ziehl Neelson Fast Green modification and examined microscopically for one hour. The precipitated protein gives a good background and the organisms are more readily seen. They are reported "acid fast rods found." Usually there are few organisms. The finding of large numbers of organisms should be viewed with skepticism and immediate checks should be started.

All of the spinal fluid received is cultured on tubes of Lowenstein-Jensen modification media for tubercle bacilli. The medium is dispensed in screw-capped tubes, and after inoculation slanted for a period of time

before incubation at 37° C. The cultures are examined every two weeks, and held for four months, and if no growth appears, are then reported—"no T.B. grown." Positive cultures are usually obtained in from four to six weeks but occasionally may need three to four months. The appearance of typical colonies of *M. tuberculosis* confirmed by direct smear examination is reported "T.B. grown." If the colonies or smears are atypical, confirmation must be done in guinea pigs.

For guinea pig inoculation, two tuberculin negative guinea pigs are injected subcutaneously in the inguinal region with spinal fluid—2cc. if available, or culture suspension grown from the spinal fluid. One pig is sacrificed at the end of six weeks and if grossly negative, the second pig is held an additional four weeks. In positive guinea pigs, generalized tuberculosis is found. Cultures and smears are made from the liver and spleen.

Whenever a smear is being examined for acid fast rods, one should always be alert for fungi. Since all our specimens are collected in autoclaved tubes, the finding of fungi on direct smear is very significant. This is the manner in which many of these organisms are first suspected since mycotic diseases of the brain simulate tuberculous meningitis. All spinal fluids for fungi are examined directly by wet smears, india ink smears, and smears stained by Giemsa's method. They are cultured on Sabouraud's glucose agar and blood agar. Four mice are injected intraperitoneally with 2cc. of spinal fluid. All fungi found in spinal fluids may be isolated in mice and this is the surest way of finding them. The mice are sacrificed one at a time, from two weeks following injection to four weeks, and the liver and spleen then cultured. The mice may die sooner and if possible are sacrificed just before death. Ether or chloroform is never used to kill the animals due to the deleterious effect upon the fungi.

Dark Field examination of spinal fluid is necessary for the finding of leptospira when the meninges are involved in leptospirosis. Cultures are made in Schüffners medium. When growth occurs the strain is identified by agglutination.

Trichinella may be found in spinal fluids. The spinal fluid is centrifuged and wet preparations examined microscopically for larvae. Only in patients with very heavy infestation would one be likely to find them in the spinal fluid.

Speed of examination, cleanliness of tubes in which the spinal fluids are collected, bedside culture of fluid into media and accuracy in reporting are most essential in the bacteriological examination of spinal fluids.

RESUMÉ OF THE C-REACTIVE PROTEIN*

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The C-Reactive Protein was first described by Tillet and Francis (1930) in patients suffering with pneumococcal pneumonia. Later its presence was noted in the sera of patients with acute rheumatic fever, Staphylococcal osteomyelitis, subacute bacterial endocarditis, infections due to Gram negative bacilli, and various other diseases.

This protein, which is not present in normal sera, apparently occurs in response to a variety of inflammatory stimuli, and may be identified by its capacity to form a precipitate with the somatic C-polysaccharide of the Pneumococcus. The appearance of this protein in the sera and other body fluids during many acute pathological conditions—both infective and non-infective—indicates that it is non-specific in its reactions. The time of its appearance (during the early acute phase) and its decline (during convalescence) is the reverse of the action of specific antibodies.

Some of the properties of this protein include:

1. CRP is a protein associated with the albumin fraction that is precipitated by ammonium or disodium sulfate between 90% and 75% saturation.
2. This protein forms a precipitate with a specific carbohydrate (C-polysaccharide of Pneumococcus) while normal serum proteins do not form a precipitate with the C-polysaccharide.
3. This protein will precipitate with the C-polysaccharide only in the presence of calcium ions, thus oxalated or citrated blood cannot be used for the test.
4. It can be separated from the normal serum albumin fraction by dialysis against tap water. (Normal serum albumin is insoluble in water, but CRP is insoluble in water containing a trace of calcium).
5. It loses its activity when heated above 65° C.
6. It is non-specific and it is not related to the etiological agent as is the case of most antibodies.
7. It is a strong antigen, which is unlike normal serum proteins.
8. Anti-serum can be prepared from rabbits immunized with CRP.
9. The anti-serum thus prepared, reacts specifically with CRP in acute phase serum and does not react with the proteins of normal human serum—has immunological specificity.
10. In the C-polysaccharide test for CRP, there is the possibility of false positive tests due to C-polysaccharide antibody, but this difficulty is not encountered with the anti-serum.
11. By means of the anti-serum it is possible to detect amounts of CRP which are too small to yield a visible precipitate in tests with C-Polysaccharide.

The presence of this protein in the blood of rheumatic patients is an extremely sensitive and reliable indicator of rheumatic fever; and it is particularly valuable in helping to establish the existance of smouldering rheumatic activity when clinical signs are equivocal. Persistance of this

* Reprinted from the Houston District Society SCOPE, August 1958.

protein during treatment with anti-rheumatic agents such as salicylates, ACTH, and Cortisone indicates inadequate therapy, and treatment should be vigorous enough to at least effect the reversal of a positive test to a negative test. The reversal of CRP during treatment does not always indicate complete suppression of active carditis. It may also reappear after the withdrawal of treatment, and if the CRP remains positive for more than two weeks it is indicative of continued rheumatic activity.

The CRP test is not of any assistance in the differential diagnosis of rheumatic fever, since it occurs in other diseases with similar clinical picture. Also, a negative test does not necessarily exclude the presence of the rheumatic process, since the various manifestations and stages may be so low-grade as to be below the threshold of an inflammatory stimulus necessary to cause the appearance of this protein. Among the isolated rheumatic manifestations which do not elicit the appearance of this protein are chorea (erythema Marginatum), subcutaneous nodules, and occasionally rheumatoid arthritis.

The disappearance of this protein is a good prognostic sign for the termination of a rheumatic attack. The sedimentation rate will often remain elevated during the period after the protein has disappeared, but the CRP has proven to be more reliable in indicating recovery.

Experiments have demonstrated that the CRP is not in any way related or responsible for the changes in the sedimentation rate. A normal sedimentation rate and a positive CRP has been encountered in rheumatic fever.

In the future the CRP test may be useful in the detection of tuberculosis, cancer, etc., since a strongly positive test has been encountered in a case of bronchial carcinoma without any indication of rheumatic activity.

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POSITIONS WANTED

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NEW TRENDS IN COAGULATION*

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The purpose of this paper is to present, in a readily digestible form, some of the applications from recent research in the physiology of blood coagulation. Clinicians and laboratory workers alike frequently state that trying to delve into the literature in regard to a bleeding patient is almost a hopeless task for anyone but the researcher in coagulation problems. Biggs and MacFarland estimate over 600 papers on blood coagulation were published in 1953-54 and the tempo is still increasing. Add to this the Babylonian confusion in nomenclature and the frequent substitution of hypothesis for facts by the leading proponents of the various "schools" of coagulation (more than 50 coagulation substances have been proposed) and some of the problems faced by the clinician and technologist can be appreciated.

It is my hope to present a workable outline which can be used for the understanding of the bleeding patient and, in his ultimate categorizing, using those tests readily available in most adequate laboratories and requiring in supplies little more than those generally available together with some ingenuity. The clinical and biochemical aspects as well as the more specialized procedures generally available only from the hematologist will be omitted.

GENERAL THEORY OF BLOOD COAGULATION. Hemostasis consists of (1) an initial vasoconstriction, (2) platelet agglutination, (3) endothelial adhesion and (4) fibrin formation. We will be mainly concerned here with fibrin formation. The so-called classical theory of blood coagulation (fibrin formation) is (1) thromboplastin plus calcium plus prothrombin forms thrombin. (2) Thrombin plus fibrinogen forms fibrin. The clotting of fibrinogen with thrombin is the only directly observable phenomenon. In spite of the vast research since this time, this theory of blood coagulation has never been challenged, but only expanded. At least five new factors have been found necessary for the formation of thromboplastin. When these are reported by different investigators and given different names, it could seem to the casual reader that at least thirty such factors must have been described. One of these factors is derived from platelets. The other four are present in normal plasma. These latter are antihemophilic globulin (A.H.G.), Christmas factor (plasma thromboplastin component), factor V (accelerator globulin, prothrombin accelerator, labile factor), and factor VII (prothrombin conversion factor, serum prothrombin conversion accelerator, S.P.C.A., proconvertin and convertin).

Figure 1 shows a simplified theory of blood coagulation based upon known facts about substances for which there is definite chemical evidence. This, as a working hypothesis for use in patient studies, purposely ignores such phenomena as plasma thromboplastin antecedent (P.T.A.), Stuart factor, CAR factor, Hageman factor, Duckert's factor X or plasma thromboplastin factor D, since it is not quite clear if indeed all these factors exist as substances. It does include factor VII which, although essential for the tissue activation of thromboplastin and the first substance decreased in patients treated with the dicoumarin group

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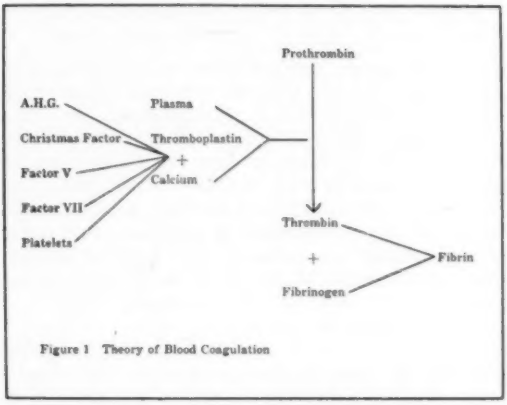

FIGURE 1—Theory of Blood Coagulation

of drugs, does not appear to be required for the blood system. It also omits, for simplification, inhibitors of coagulation, although these will be discussed later.

GENERAL TESTS OF CLOTTING FACTORS. The necessary laboratory tests for the identification of coagulation disorders must include: (1) whole blood clotting time, (2) thrombin generation test, (3) one stage prothrombin time (Quick test), (4) two stage prothrombin time, (5) serum prothrombin consumption, (6) thromboplastin generation and (7) fibrinogen determination.

(1) **Whole Blood Clotting Time.** This test consists of placing a volume of blood into glass tubes and measuring the time it takes for a clot to appear. This test is influenced by a multitude of variables which means it cannot be used for recording small differences. It remains useful in that 99% of patients with clotting times greater than 30 minutes (normal 5-10 minutes) will have hemophilia. It is a good gross test.

(2) **Thrombin Generation Test.** This essentially consists of removing aliquots of clotting whole blood (or, in the modification of Pitney and Dacie, citrated plasma recalcified) at regular intervals, and measuring the time necessary to clot fibrinogen. This test is nonspecific diagnostically, but is valuable in predicting a tendency to bleed. In other words, it is an extremely sensitive form of securing the same information as from the whole blood clotting time.

(3) **One Stage Prothrombin Time (Quick Test).** This is the usual test used for determining response to the coumarin drugs therapeutically. Diagnostically, prolongation of the prothrombin time is found in deficiencies of factor V, VII, fibrinogen or prothrombin. To separate these substances, additional substances can be added and the test repeated. This is demonstrated in Figure 2. Storage of oxalated plasma lengthens the time while storage of citrated plasma shortens it.

(4) **Two Stage Prothrombin Time.** Tissue thromboplastin (lung,

FIGURE 2—Differential Diagnosis by Adding Substances (See Test)

	V	VII	Proth.	Fibrinogen	Circulating anticoagulants
$BaSO_4$ Adsorbed Plasma	will correct	will not correct	will not correct	will not correct	will not correct
Normal Serum	will not correct	will correct	will not correct	will not correct	will not correct
Fibrinogen	will not correct	will not correct	will not correct	will correct	will not correct
Normal Plasma	will correct	will correct	will correct	will correct	will not correct

brain, etc.) plus calcium chloride are incubated with plasma for a given period of time, the clot removed, and the remaining plasma tested for the clotting time with fibrinogen. If varying periods of time are used, a table can be made indicating the amount of thrombin formation from which the prothrombin content can be determined. In practice, I use this method for the plasma control of the serum prothrombin consumption test and use a routine one-minute incubation time.

In all cases where a tissue thromboplastin and calcium chloride are required I have found Simplastin (Warner-Chilcott) to be satisfactory.

(5) **Prothrombin Consumption.** This test is essentially the two stage prothrombin time performed on serum one hour after clotting, and measures the amount of thromboplastin generated indirectly by measuring residual prothrombin complex. Normal is 2½ times or more the plasma time. Constant controls must be run, but the test is reliable, fairly sensitive and easy to carry out. A decreased time indicates a deficit in A.H.G., Christmas factor, platelets or (questionably) factor V. This may be set up as an "index" by dividing the plasma time by the serum time and multiplying by 100. Normal prothrombin consumption index is then less than 40. It is frequently greater than 100 in hemophilia. It will be abnormal in approximately 95% of cases of hemophilia.

(6) **Thromboplastin Generation Test.** This is the most sensitive method for measuring defects in thromboplastin formation, and may be the only abnormality found in mild cases of factor V deficiency, Christmas factor deficiency, hemophilia and thromboasthenia. It is based upon the finding that if normal plasma is adsorbed by barium sulfate (or aluminum hydroxide) prothrombin, factor VII and Christmas factor are removed, leaving antihemophilic globulin and factor V. If to this plasma is then added normal serum (which contains factor VII and Christmas factor), platelets and calcium chloride, thromboplastin is formed. This thromboplastin generation can be followed by subsampling from the mixture into normal plasma (with calcium chloride) and measuring the speed of clot formation. By substituting for normal plasma,

serum or platelets, the same materials from the patient's blood, the differential of these factors can be made. Thus, if barium sulfate adsorbed plasma for the patient, together with normal serum and platelets gives an abnormal result, then the defect is Hemophilia or (rarely) factor V deficiency (since factor V is supplied with normal platelets). If the patient's serum (other factors from normal blood) gives an abnormal result, then the patient has Christmas disease. If the patient's platelets (other factors from normal blood) results in a prolonged clot time, the defect lies in the platelets. If the patient's adsorbed plasma and platelets together with normal serum gives an abnormal result, then we have factor V deficiency.

(7) **Tests for Fibrinogen.** This may be determined biochemically or roughly with the use of Fibrindex. In addition, the Lee and White clotting time and the one stage prothrombin time are abnormal in fibrinogen deficiency. The clot is either entirely absent or only a very tiny clot forms. The usual laboratory finding in afibrinogenemia is complete absence of clotting by any of the usual tests.

The differential diagnosis of coagulation defects based on these laboratory tests is summarized in Figure 3.

FIGURE 3—Differential Diagnosis of Blood Coagulation Defects Based on Usual Laboratory Tests

Factor Deficiency	Clot Time	Thrombin Gener.	One Stage Proth. Time	Two Stage Proth. Time	Proth. Consumption	Thromboplastin Gener.	Comments
Fibrinogen	↑*	—	↑*	—	—	—	Biochemical Methods. Poor Clot
A.H.G.	↑	↓	—	—	↓	↓*	See also under thromboplastin generation for differential diagnosis
Christmas	↑	↓	—	—	↓	↓*	
V	↑	?	↑*	—	↓	↓*	
VII	—	—	↑*	—	variable	—	See Figure 2
Prothrombin	↑	↓	↑	↑*	—	—	
Platelets	—	↓	—	—	↓	—	Platelet count. Clot retraction

* = Test most sensitive for minor deficiency.

INHIBITING AGENTS. It should be obvious that in order for the blood to remain fluid in the vascular tree there must be mechanisms to prevent coagulation, or in the case of intravascular clotting (thrombosis) to prevent extension of this process, even though the essential factors for coagulation are present. These substances are referred to as inhibiting agents and in certain situations become increased in amount or additional substances (Dicoumarol, Heparin, etc.) may be given to patients to make spontaneous coagulation more difficult. To date the only proven naturally occurring inhibitor of coagulation is antithrombin. Some notice of its powerfulness is provided by the observation that the thrombin formed from 10 ml. of plasma would be more than enough to clot all the blood in the body were its action unopposed. Another substance present in body tissues is heparin, which prevents the conversion of prothrombin to thrombin (? antithromboplastin) and, together with an albumin cofactor, delays the reaction of thrombin with fibrinogen to form fibrin. In addition, Tocantins has isolated an antithromboplastin. Besides these naturally occurring anticoagulants (which may increase pathologically under certain stimuli), others such as anti-A.H.G. may occur is a "primary" hemorrhagic disease or secondary to pregnancy, multiple transfusions, etc. The identification and measurement of these anticoagulants can be made by means of various tests not generally available in the average hospital laboratory, such as the method of Astrup and Daily for antithrombin activity, the measurement of heparin by the method of Jaques and Charles, the effect of tolouidine blue, etc. In general, to demonstrate the presence of an anticoagulant it is only necessary to mix a small quantity of the test plasma with normal plasma, incubate and measure either the clotting time after the addition of calcium or to use the same mixture of plasma after barium sulfate adsorption and use the thromboplastin generation test on the various mixtures.

This discussion has centered around the factors necessary for fibrin formation. Although platelets are generally included in such a discussion, it is quite doubtful that platelet deficiency to the degrees found in clinical situations would cause serious hemorrhage if it were not for associated capillary damage. Hemorrhage due to thrombocytopenia or thromboasthenia is generally of a different type than that due to coagulation disorders involving a deficiency of one of the necessary factors. In general it is more likely to be oozing, petechial or purpuric and to involve mucous membranes, skin structures and deep tissues. In general, these conditions are characterized by a prolongation of bleeding time and, of the tests discussed, thrombocytopenia affects only the thromboplastin generation and prothrombin consumption tests.

Summary

A brief outline of the tests necessary in differentiating the causes of deficient fibrin formation has been presented. Using these tests more than 99% of the cases should be able to be categorized and thus the necessary therapy given.

SOME LABORATORY METHODS USED IN THE DIAGNOSIS OF FUNGUS DISEASES†

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In the last few years considerable progress has been made in the development of laboratory methods for the diagnosis of deep fungi. The medical technologists rightly can be proud because two of the most helpful procedures for the diagnosis of fungi in tissues have been developed by fellow technologists. I refer to the Gridley[3]* procedure and the Grocott* modification of the methenamine silver stain of Gomori. Both give excellent results and have contributed to the recognition and understanding of pathologic changes caused by fungi as much as anything developed in a technical respect during the last 20 years. The Gridley procedure has certain difficulties because it requires extreme cleanliness in the preparation, preservation of some of the reagents in the dark or under refrigeration and renewal of some of the solutions rather frequently. On the contrary, the Grocott procedure is technically easy and gives excellent results. Occasionally in cases which are negative with the Gridley stain one can find abundant fungi with the Grocott procedure and therefore many workers in the field use it with preference. In addition the Grocott stain produces excellent preparations for photography. It is very useful always to run a positive control in order to be certain that the proper reaction has been elicited. We use for this positive control animal tissues generally, since it is no problem to obtain a block from infected animals and to cut a hundred slides, one of which is used as a control each time the stain is utilized. Personally, we believe that both Gridley and Grocott stains are very much superior to the periodic acid-Schiff procedure which is still used in many laboratories.[5] Considerable experience is necessary to recognize the type of fungus with which one is dealing. It should be kept in mind, however, that only a positive culture gives an absolute diagnosis. There is always the possibility of an error to be considered if only morphologic methods are taken as the base for diagnosis. Due to the extensive use of special stains we have learned in the last years that the variation in the size and shape of fungi is very much larger than textbooks lead us to believe. A table showing the variations observed in this laboratory clearly indicates the great danger which the inexperienced observer may encounter if he would take size alone as the criterion for diagnosis. This leads us to a discussion of the importance of cultural methods since a positive culture leaves no question about the identity of the organism. Innumerable culture media have been introduced, used, and rejected in the past years in part because they are hard to produce and reproduce, in part because the average clinical laboratory uses mycologic media so seldom that they are not fresh when the occasion arises. We therefore, restrict our cultural efforts mostly to Sabouraud's glucose-agar which we use in screw-top perfume bottles.** These permit storage for many

* Miss M. F. Gridley was a leading tissue technician in the Armed Forces Institute of Pathology in Washington, D. C., where she compiled the "Manual of Histologic and Special Staining Technics" edited in 1957.

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** Owens Glass Company, Toledo, Ohio.

† Reprinted from The Bulletin of the Ohio Society of Medical Technologists, August 1958.

SIZE OF YEAST CELLS IN TISSUES
TABLE 1

Organism	Average Size	Extreme Sizes Recorded
Candida albicans	3 - 6	1 - 7
Cryptococcus neoformans	5 - 15	3 - 20
Histoplasma capsulatum	2 - 5	1 - 20
Blastomyces dermatitidis	7 - 15	2 - 30
Blastomyces brasiliensis	10 - 30	4 - 80

months without drying out of the medium. Some authors object to the use of a screw-top bottle because it is supposed to produce a semi-anaerobic condition. However experiments in this laboratory have shown that sporulation and growth are not at all inhibited under this condition.[1]

Certainly in certain situations one will use blood-agar which is available in all bacteriologic laboratories. It is an excellent medium for the culture of most yeast-like organisms. If the material to be cultured is not contaminated; i.e. pus obtained by aspiration, pleural fluid, spinal fluid, abdominal fluid; then we inoculate it directly onto media which contains 40 units of penicillin and 20 units of streptomycin (to suppress the growth of bacteria). If, however, the material is contaminated with bacteria; i.e. sputum, urine, bronchial washings, drainage from sinuses, etc., then we mix the material with equal amounts of antibiotic solution containing approximately 2,000 units each of streptomycin and penicillin per cc. and inject 1 ml. of the mixture intraperitoneally into two hamsters. We have found that the hamster (Cricetus auratus) is much more susceptible to histoplasmosis and other fungus diseases than the mouse.[6] The initial cost is approximately twice that of mice but this is minimal when one considers the greater yield of positive results.

The hamsters are kept for four weeks. They are then killed with chloroform and pinned to a paraffin tray. The fur is moistened with alcohol. We wait religiously until the alcohol evaporates otherwise it will be carried into the abdominal cavity, and the cultures will become sterile. We open the abdomen with a V-like incision, the apex being in the region of the symphysis pubis of the animal and the ends of the V in the region of the costal margins. This permits a flap consisting of skin and peritoneum to be reflected without touching the liver and spleen with the instruments. Then we change instruments (which have been sterilized) and take small pieces of liver and spleen from each animal. These are put directly onto Sabouraud's agar and smeared on the surface without bothering to mince them first. The results of this procedure are very satisfactory and frequently after only 6-10 days one sees beginning growth. It seldom takes more than two weeks until fairly well-developed colonies can be recognized on top of the small pieces of liver or spleen. Mounts are made, identification is established, and the case is concluded.

At the time of the autopsy of the animals, small pieces of liver and spleen are taken for microscopic examination.

We have learned to include the hilus of the spleen where lesions frequently can be found after intraperitoneal injection even if no

pathology is found in the rest of the organ. Gridley and Grocott stains are done automatically from the hamster tissues if positive findings are suspected. Frequently at autopsy of the hamster, grossly recognizable lesions are absent. In other words, hepatosplenomegaly may be present in some cases, but its absence does not rule out a positive cultural result from liver or spleen. The combined program of injecting animals and culturing from the infected hamster takes a minimum of 6-8 weeks, which indeed is, in some cases, too long to be of much value for clinicians. However, at present we have no better or faster methods to get results from contaminated material. Some mycologists add actidione to the media which is supposed to suppress saprophytic fungi.[2] However, actidione has several drawbacks, particularly its selective suppression of Cryptococcus neoformans. Vary obviously in clinical laboratories one will frequently be at a complete loss to know what fungus is suspected by the clinician. Therefore actidione is not the ideal solution for avoiding overgrowths by saprophytes in that it eliminates one of the more important fungi as well. It has been our experience that passage through animals is the safest and in the long run, the shortest method to obtain positive results.

Summary

Special stains are an absolute necessity for the demonstration of fungi in tissue.

Morphologic pitfalls in the diagnosis of mycotic diseases should be avoided by cultural methods.

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IF I WERE AN ACTIVE MEDICAL TECHNOLOGIST AGAIN*

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If I were an active medical technologist again I would try to excel. A few of us seem to think it isn't worth the effort and somehow unseemly to shine in our role in the laboratory. Is this not effrontery in the midst of so much scientific knowledge and brilliance? No attitude is more damaging to the position of the individual medical technologist, nor to his colleagues in the field, nor more importantly, to the service he can perform for society.

This feeling of inferiority is as harmful as the opposite pole of brashness and self serving. It is time to stop equating the anonymity of the medical technologist with insignificance and servitude. It is time he forgot about anonymity altogether and became recognized for what he is—one of the most important members of the medical team in any institution.

Our inferiority very often is caused by our lack of knowledge. How many of us bother to read the scientific journals, buy a new book, or even bother to borrow from our hospital libraries? A well known pathologist, with whom I once worked, said that medical technologists will buy a couple or more hats a year but not think of buying a new scientific book. Another well known pathologist said he had the latest books and journals in his library which should be made available to his technologists but he couldn't seem to get around to loaning them. This pathologist would no doubt be surprised but very pleased if his technologists took the initiative and requested the books.

I would try to truly assist my pathologist, not just work *for* him but *with* him. I know excellent technologists who keep their pathologists informed of the latest techniques by doing much of the reading that pathologist does not have time to do. Pathologists can't be expected to know the procedures for each laboratory test and very often they haven't found the time to study the theory of a new technique. But a good technologist is there to brief him if necessary, Technologists capable of giving expert assistance to their pathologists command their respect and are usually liberally compensated.

I would consider it my professional duty to accept responsibility even though it would mean longer hours and continual study. Many medical technologists have the formal education and training for continued and extensive study. Eighty-three percent of all registered medical technologists have at least a baccalaureate degree.

If I were an active medical technologist again, I would take pride in my profession. If you don't take pride in those tasks you do well, how can you expect others to recognize the value of your work and to treat you as a professional.

I would concern myself with upgrading, in a tangible way, that most intangible of all things—my profession's reputation. For it is *reputation* that determines the kind of people that this profession interests. And con-

* From the Summer 1958 issue of the Journal of the Empire State Ass'n of Medical Technologists.

versely it is the people who go into medical technology who determine its reputation.

Complacency and mediocrity are more and more difficult to camouflage. Competence and excellence are in greater demand, and the search for them is more persistent and thorough.

I would work for the constant upgrading of the medical technology profession, not only locally, but nationally. I would believe in it and *work for it*, or I would get out of it.

I would be proud to be asked to join my professional societies and would join them at the earliest opportunity. Any professional society, if it is to be effective, must truly represent the majority of those practicing the activity involved. This is why, for example, such organizations as the American Medical Association and the American Institute of Certified Public Accountants are as effective as they are. They do represent their respective professions.

The American Society of Medical Technologists represents the largest organization of members of our profession in the world,, and it is steadily growing larger. But at this time it does *not* adequately represent the majority of that group which practices medical technology. Less than half our number belong to their professional society, either because the idea never occurred to them or because they do not understand the importance of joining.

By joining my professional society I would consider it my duty to take an active part in its functions and to do everything I could to help the profession gain recognition.

There are some things I would not do—if I were an active medical technologist again.

I would not work as an isolationist. I would try to make use of the technical knowledge of the best minds in my profession and related fields, consulting and studying with them.

I would not join the chorus of moaners and groaners. Medical technologists never had it so good. They are in great demand and the medical team can't get along without them. Today they have stature in their institutions and in the medical family. Salaries are rising and will continue upward. Wishing won't make it so, but competence and hard work will. With all the new advances in the science of medicine, the time is stimulating and exciting. Medical technology work was never more attractive.

If your pay and working conditions are not all you think they should be, remember that they have improved sufficiently to encourage what is nothing less than the birth of a new profession. The American Society of Medical Technologists celebrated only its 25th Anniversary two years ago. There are more than 8,000 members today.

What would I do if I were an active medical technologist again? I would try to become the ideal medical technologist I have described earlier in these remarks. I would try to do the same things I used to do—but I would try to do them better.

WHAT BETTER SCHOOLS WILL MEAN TO RECRUITMENT*

Panel Program:

Program of Approved School Directors and Teaching Supervisors to Improve Educational Methodology Throughout Alabama.

One year ago in Alabama there was held a meeting of Approved School Directors and Teaching Supervisors interested in recruitment and related activities. The common problem which motivated this group was the need for more students in the schools of medical technology of our state. Means were sought for stepping up our recruitment efforts through increasing personal contacts with students and through improving schools of medical technology. Two plans of action emerged from the discussion of this group and two committees were set up to activate these plans. First the Alabama Inter-Society Committee for Careers in Medical Technology was formed. This committee is concerned with recruitment and related activities. Each of the organizations represented has included a provision for this committee in its By-Laws. A second result of this meeting was the formation of a permanent organization of teaching supervisors with annual or semi-annual meetings. It is notable that all these Teaching Supervisors are Registered Medical Technologists (ASCP) and they actively participate in their state and national professional societies. This group is aware of the relation of improved schools of medical technology to long term recruitment. We are concerned with improving the performance of Medical Technologists who are at present teaching in our hospital schools and with providing teachers for the future. The quality of the hospital training will give the school a good or bad reputation which will inevitably reflect upon the recruitment in the area, either discouraging or attracting students.

In March 1958, the Teaching Supervisors met for the first time. The purpose of this meeting was to exchange notes, ideas and visual aids used in teaching programs in the various schools represented. Each person attending brought with him nine copies of his notes in one department. Subjects had been assigned so there was no duplication. Exchange of this lecture material served as a basis for comparison of teaching methods in the schools represented. The participation was enthusiastic. The group agreed that the ability of the graduate medical technologist to obtain good, accurate results "under fire" is the foremost criterion for judging the school from which he came. In order to produce professional workers who can perform with skill and versatility in the many departments of a clinical pathological laboratory, the school must provide adequate instruction and opportunity for experience. The schools of medical technology have evolved from the old apprenticeship type of teaching. Here practical instruction was given at the work bench and ample opportunity for participation was offered. The "how" of a technique was learned well, but the "why" often unexplained or misunderstood.

The increasing complexity of the procedures used in the clinical laboratory makes the improvement of our teaching of medical technology imperative. Most of our teachers are technologists who have been made

* By Mrs. Sarah Crowson, Birmingham, Alabama.

teachers of necessity but who do not have a knowledge of educational methodology. They have become teachers because they have knowledge and enthusiasm and a desire to teach. Teaching skills must be acquired. Our Alabama Teaching Supervisors felt that they needed instruction in teaching methods.

For our second meeting October 3, 1958, two speakers were invited. The Very Rev. Brian Egan, president of St. Bernard College, St. Bernard, Alabama, spoke on "Teaching Methods." Father Egan analyzed the three techniques of teaching: the problem technique, the participation technique and the practice technique, in their relation to the mental process, the learning process and the curriculum. The second speaker for this meeting was Major Thomas G. Ellis of the U. S. Air Force, Gunter Air Force Base, Alabama. Major Ellis discussed "Compilation of Laboratory Procedures for Teaching Notebooks." He gave us much practical instruction from his broad experience in compiling teaching manuals for the U. S. Air Force and from their visual aids workshop. After this rewarding and stimulating program each supervisor talked for five minutes on methods for improving laboratory teaching programs. Some of the topics discussed were: how to outline the physical needs of the school of medical technology and the development of its budgetary resources; the importance of having students attend clinical pathological conferences of the staff physicians; and the importance of giving each student a project which will involve some research.

Long term projects undertaken by this group are: 1) compiling a laboratory teaching notebook and 2) making a collection of visual aids. The group feels that in order to improve educational methodology throughout the state we need a state educational director.

In May 1958, the Alabama Society of Medical Technologists adopted the following resolution: "Be it resolved that the Alabama Society of Medical Technologists in session May 3, 1958, request the National Committee for Careers in Medical Technology that Alabama be used for a pilot study to determine the scope of activities of an educational director. The Alabama Society recommends: 1) that a grant be obtained by the NCCMT for making the pilot study in Alabama; 2) that a teaching supervisor on loan from an Alabama School be used to make the study; 3) that teaching supervisors and pathologists directors in Alabama Schools cooperate fully in this pilot study and 4) that results of this study be used to determine the advisability of having a National Educational director.

Future plans of the Alabama Teaching Supervisors include inviting faculty members from affiliated colleges to participate in these meetings and cooperation in sponsoring Medical Technology Clubs on campus. Their activities will improve the liaison between the hospital schools and the campus. We may expect a resulting decrease in drop-outs during the two to four years that the students spend on the campus.

When the broad aspects of the problem of medical technology education are considered it appears that state and regional meetings of pathologists, directors and teaching supervisors to discuss problems and consider solutions will result in cooperative action. We have seen the stimulus derived from such meetings and would recommend this to you as one way of improving educational methodology in the hospital schools.